THE NUTRITIVE VALUE OF GUAR MEAL

(*Cyamopsis tetragonoloba* L.) FOR POULTRY

By

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TO MY PARENTS

DECLARATION

This thesis describes the results of research carried out by me, unless otherwise stated, at the Agricultural Research Council's Poultry Research Centre, Edinburgh, during the period between October 1974 and September 1977.

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ABSTRACT

Three commercial samples of guar meal were analysed for their proximate components, minerals, amino acids and the presence of certain toxic substances.

The values of these guar meal samples in diets for broiler chicks and laying hens were examined in several small and large-scale experiments.

The effects of heat processing the meal, supplementation with methionine, hemicellulolytic enzymes or cholesterol, and extraction with ethanol or dilute alkali on its nutritive value for poultry were studied.

The presence of a toxic saponin in guar meal has been demonstrated by both chemical and biological tests.

It is concluded that the presence of this saponin fraction and the residual gum are the major factors responsible for the poor performance of poultry fed diets containing guar meal.

However with suitable processing, supplementation with limiting amino acids and the addition of enzymes, the performance of birds fed guar meal has been significantly improved. The nutritive value of the guar meal protein is estimated to have been improved four fold as judged by net protein utilisation and it is thought there is scope for a further small improvement.

More complete removal of the gum and inactivation of the saponin are likely to allow guar meal to be accepted as a high protein ingredient for inclusion in poultry diets.

1. INTRODUCTION

1.1 The guar plant

Cyamopsis tetragonoloba L. (syn. *Cyamopsis psoraloides*), a member of the family *Leguminosae*, sub-family *Papilionaceae*, is a drought-tolerant annual herb indigenous to the Indian subcontinent. In the English language it is commonly known as guar. This vernacular name is derived from the Sanskrit word "go" or "gav" which means "cow" (Monier-Williams, 1956). It is now popularly known as guar in India although slight variations in spelling and pronunciation occur from region to region. Some less common English names for guar are: cluster bean, aconite bean, Calcutta lucerne, field vetch, four-angled bean and Siam bean.

The guar plant is a hardy summer-grown legume which thrives best on lighter soils (e.g. sandy loam) under climatic conditions similar to those required by maize, sorghum, pearl millet, cotton and groundnuts. Requiring very little water for growth, guar is a sun-loving plant which, nevertheless, sets abundant seeds when grown in arid areas under irrigation. Humid conditions, however, delay maturation of the seed and rain falling on the crop after it has matured causes the seeds to turn black and reduces their feeding value. The plant has an upright and branching growth habit, is self-supporting and reaches between 1 and 2m high at maturity. The tiny rose-coloured flowers which grow in clusters at the axils of the leaves are the precursors of the pods. It is in these leathery pods that the guar seeds, up to about ten in number, are contained. The guar plant, its pod and seeds are shown in Figure 1.1.

Like other members of the *Leguminosae*, guar is able to thrive upon ground which is devoid of combined nitrogen. Through symbiosis with bacteria of the *Rhizobium* species, atmospheric nitrogen is oxidised and used by the plant. Oke (1967) noted a

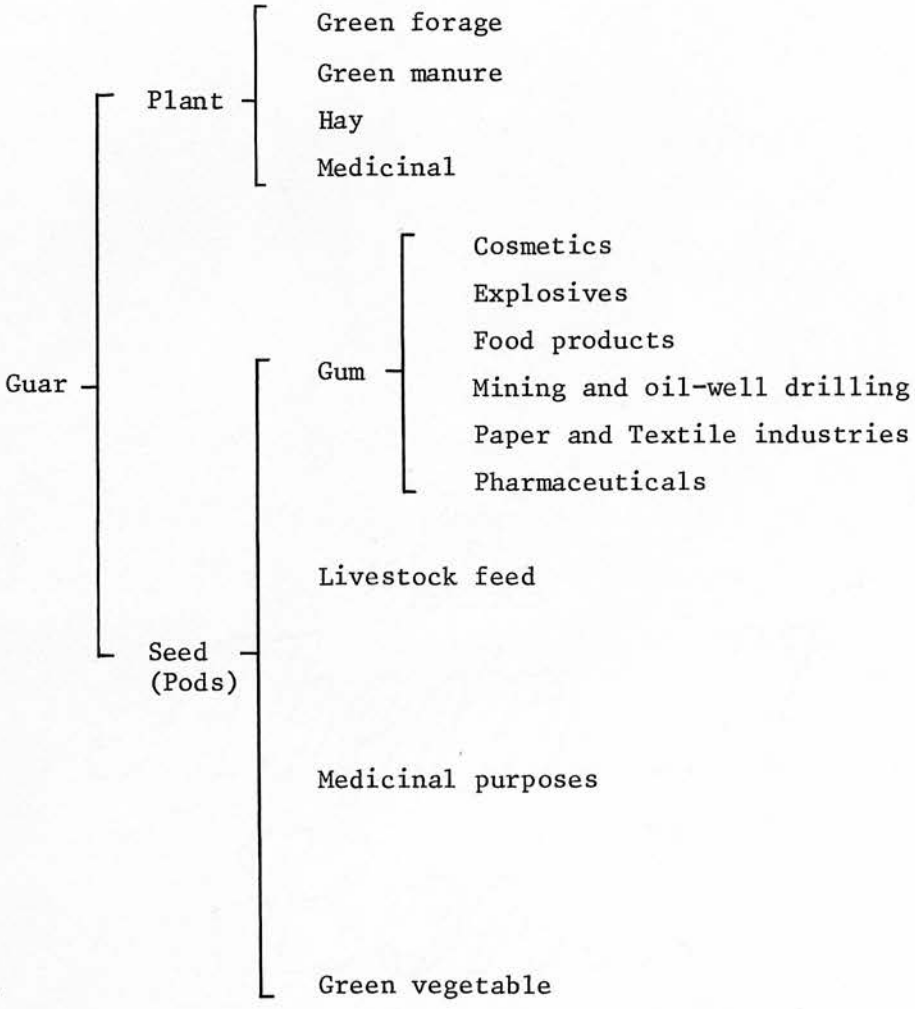
Guar plant, pods, and the seeds

Fig. 1.1 1. A typical field of guar crop. 2. Guar plant showing leaves, pods and flowers. 3. Mature pods and seeds.

steady increase in the nitrogen-fixing capacity as the guar plant matured, a maximum of approximately 5 mg nitrogen/day being reached towards the end of the growth phase. Thus, provided that nodulation is effective in supplying the plant with nitrogen, added fertilizer nitrogen affects neither the seed yield nor the basic structure of the plant (Sanderson, 1974). Coupled with its drought-tolerance this remarkable ability to enrich rather than deprive the soil of nitrogenous compounds has made guar a multipurpose choice crop for many of the drier areas of the world, especially those where agriculture is still largely dependent on nature. During extreme drought, when other crops perish, guar is likely to survive and provide the farmer with at least one source of income. It is partly because of these agronomic attributes that guar has continued to be grown and many uses have now been established for this crop (Figure 1.2).

Fig. 1.2

Uses of Guar

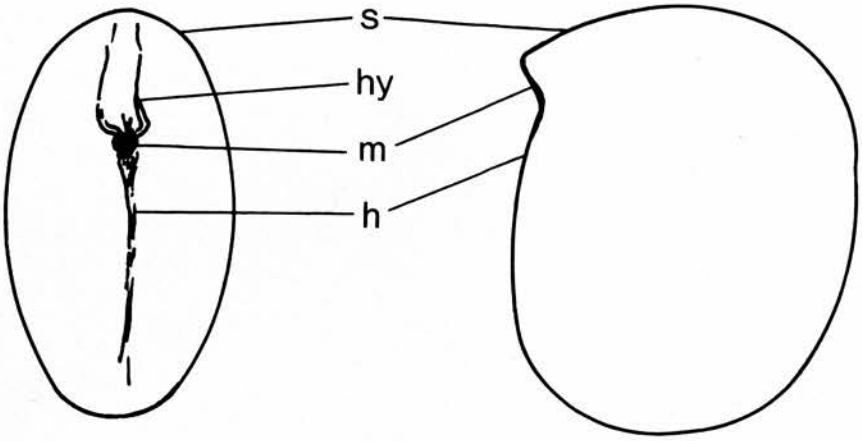


1.2 The guar seed

The mature guar seeds, as they emerge from the pods during threshing, are dry and extremely hard with a lightly blistered surface. They are small and oval-shaped, measuring about 4-5 x 5-6mm. The thousand seed weight of mature berries ranges from 30 to 50g. The colour of the seed coat, which varies from black to dull white, appears to be under environmental rather than genetic control, rain or high humidity during maturation resulting in a darker seed coat.

The guar seed is dicotyledonous and consists of three main components, testa, endosperm, and germ. The testa or seed coat is the outermost covering of the seed and accounts for most of the crude fibre and mineral matter. The germ consists of a tiny embryo bearing laterally at its midpoint two massive cotyledons. Most of the protein and oil present in the seed is contained in this fraction. Outside the germ and adhering to the inside of the seed coat is the endosperm which, almost exclusively, consists of a polysaccharide gum, the reserve carbohydrate for the new seed. The components are depicted diagramatically in Figure 1.3.

THE GUAR SEED



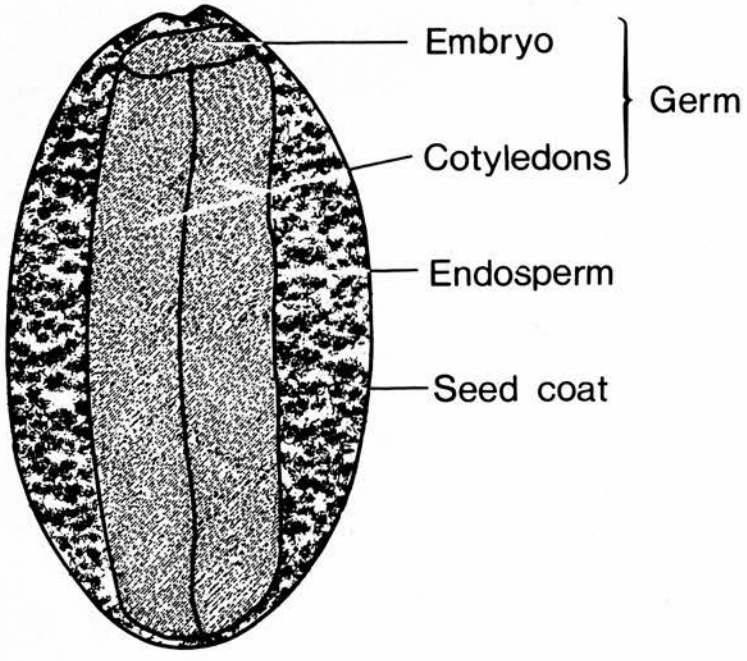


Fig 1.3. Drawing of Guar seed. Structural features are, s, seed coat; hy, outline of hypocatyl under seed coat; m, micropyle; h, hilum or seed scar.

1.3 Guar as a crop

Guar has been grown extensively in certain parts of the Indian subcontinent for many centuries where it has served local inhabitants both as a feed for livestock and as an inexpensive table vegetable. According to the American botanist, Edgar Anderson, guar is probably one of the oldest crops known to man (Goldfrank, 1957).

Today India is the largest producer and several million acres of guar are planted in the States of Rajasthan, Punjab, Gujarat, Haryana and Uttar Pradesh. An estimate of the amount of guar produced for seed purposes in India in recent years is given in Table 1.1. In addition Pakistan produces nearly 45,000 tonnes of guar and exports about 17,000 tonnes of guar products annually (Hussain and Manzoorullah, 1963).

Although native to the Indian subcontinent, guar has been introduced to many other areas of the world where suitable agro-climatic conditions exist for its cultivation. It is now grown in Western Nigeria (Opeke, 1964), Australia (Moffet, 1965), South Africa (Sellschop, 1967), and Rhodesia (Sanderson, 1974). It was introduced to North America as early as 1903 by the US Department of Agriculture when test plantings were made in Texas, Arizona and California; it did not, however, establish itself as an economically viable crop until the 1950's (Esser, 1956). In spite of these early difficulties guar seems to have adapted well to the drier areas of the south-west USA as a multipurpose crop where, by the late 1950's, 20,000 to 30,000 acres were being planted annually (Poats, 1960).

Table 1.1

Estimated area and production ('000 tonnes) of guar for seed purposes in India

State	Y E A R 1968-69	1969-70	1970-71	1971-72	1972-73	1973-74	1974-75
Gujarat	48.3	37.9	118.1	131.6	101.2	93.7	93.7
Haryana	36.5	61.7	68.0	53.9	97.8	75.9	83.8
Orissa	0.3	0.3	0.1	1.0	-	-	-
Punjab	58.5	66.7	16.2	42.9	123.3	132.7	95.6
Rajasthan	100.9	230.7	430.8	244.2	94.7	414.8	105.3
Uttar Pradesh	14.2	14.2	17.8	43.3	43.3	43.3	43.3
All India	258.7	411.5	651.0	516.9	460.3	760.4	421.7
Area under seed production ('000 hectares)	990.0	1089.8	1465.3	1538.6	1184.2	1709.5	1247.7

Source: Directorate of Economics and Statistics, Ministry of Agriculture, Government of India, 1976.

1.4 Guar gum and the meal

Until the middle of the twentieth century guar was a minor crop grown in the arid and semi-arid zones of India and Pakistan primarily for fodder production and green manuring. Surplus crop was harvested for seed production to meet the requirement for the next sowing. The remaining seed was utilized in concentrate mixtures for cattle and buffaloes. However, during World War II the importance of guar as a valuable source of gum was recognized.

Before the war, carob seed (Ceratonia siliqua) from the Mediterranean was imported into the US as a source of industrial gum (Rol, 1959). During the war, when the supplies of imported carob seed were cut off, a search for domestic sources of gum started. In 1943, a private company, General Mills Inc., initiated studies on the milling of guar seed for its endosperm. At the same time the use of guar gum in the manufacture of paper was under investigation. The results of these studies are summarized by Rowland (1945) as follows: "The beneficial effects of guar mucilage in the paper processing were sufficient to justify the adoption of the product for regular manufacturing formulas".

Having established the suitability and the utility of guar gum as a substitute for carob seed gum, interest in the cultivation of guar in the United States, as well as in India and Pakistan, was renewed. The two pioneer companies in this field in the US - General Mills Inc., and Stein Hall and Co., Inc. - set up guar gum extraction plants. By giving assurances to the growers that all the guar seed they could produce would be bought, production was put on a sound commercial basis.

Extraction of gum from guar seeds is done by a dry milling process in which advantage is taken of the differences in the hardness

of the various seed components (hull, endosperm, and germ). Purification is accomplished by multistage grinding and sifting processes. The various steps involved are shown in Figure 1.4. Because the primary object of guar seed milling is to extract the gummy endosperm, the remaining material obtained as a byproduct is termed "guar meal".

Following the installation of milling plants guar gum has been in commercial production since 1953. Exact figures for the quantities marketed in various countries are not available but estimates of its consumption in the United States from 1954 to 1960 are given in table 1.2.

From these figures it is evident that consumption of the gum in the USA increased steadily year by year and there is no reason to doubt that the growth rate has been maintained into the 1970's. The increase reflects its wide applicability and acceptability for a variety of industrial purposes.

In the US alone a much greater market potential for guar gum was recognized almost 20 years ago (Poats, 1960), when it was predicted that consumption could reach 100 million pounds per year. The need to increase the acreage over a wider geographical area to reduce the possibility of insufficient harvest in poor seasons cannot, therefore, be overemphasised.

The extraction of gum from the guar seeds results in the production of guar meal, and with demand for gum increasing it is axiomatic that increasing amounts of the byproduct will become available. Although the gum has a ready market, the meal, in spite of its high protein content, has not yet enjoyed the same popularity. Its most likely role undoubtedly is as an ingredient in compounded animal feeds as a partial replacement for the protein-

Fig. 1.4

Scheme showing the extraction of gum from guar seeds

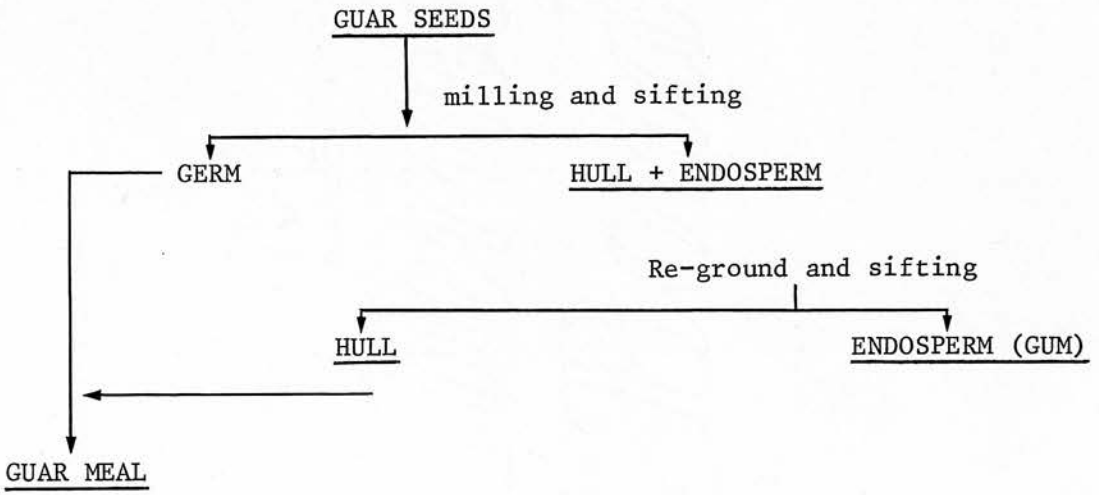


Table 1.2

Estimated consumption of guar gum in the US

Year	Amount (million pounds)	Reference
1954	2.5	Whistler and BeMiller (1959)
1955	5.5	"
1956	12.0	"
1957	15.0	"
1960	22.0	Goldfrank (1960)

rich ingredients such as soya bean meal. However problems of palatibility and nutritional shortcomings, some identified, some still unknown, have restricted its inclusion into diets for poultry to almost negligible proportions.

2. CHEMICAL COMPOSITION

2.1 Composition of guar seed

To formulate nutritionally adequate diets for livestock it is necessary to know the chemical composition of the constituent feed ingredients. For this purpose proximate analysis is generally considered sufficient to define the nutritive characteristics of a food. However, detailed analyses in respect of the amino acids and minerals are more and more being recognised as invaluable aids.

Published data from several laboratories for the organic and inorganic constituents of guar seed and meal are available. Although there are several varieties of guar grown e.g. Brooks, Groehler, Hall, Mills, Sadabahar, Texsel etc., no distinction appeared to exist in the trading of guar seeds on a varietal basis and, therefore, no attempt has been made in this study to distinguish the data according to variety.

For ease of comparison all analytical data are expressed on a dry matter basis and, therefore, were recalculated if the original data were presented on some other basis. Certain analyses which contained values for one or two constituents only were not included.

Data on the proximate composition and mineral matter of guar seed are presented in table 2.1. It will be noted that the seeds contain about 30% crude protein and are therefore classed as a protein-rich feeding stuff. The oil content, about 4%, is a useful source of energy.

Unlike other members of the Leguminosae, nearly all the reserve polysaccharide in the endosperm of guar seed is present as the Mucilaginous guar gum. This variation from the usual appears to be a phenomenon of normal plant metabolism.

There is good agreement among the analytical values reported by various workers (Table 2.1) especially when it is likely that the composition will be affected by variety grown, soil conditions, climate, and degree of ripeness (Woodworth et al., 1952; Esh, et al., 1959; Gupta and Das, 1961).

Co-operation between breeders and nutritionists in the development of improved plant strains is aided when the nutritionist can give the breeder general aims. The relative proportions and compositions of the testa, endosperm and germ have therefore been determined. The results of the analyses for crude protein, ether extract, crude fibre, ash, calcium and phosphorus are set out in table 2.2.

It will be seen that the germ (embryo plus cotyledons) forms about 45% of the dry seed weight and contains most of the protein, oil and phosphorus. The testa is low in all constituents; therefore, the feeding value of the meal for poultry could be enhanced by marketing the germ and testa separately. The endosperm is unlikely to have any feeding value for poultry because most of it is the Mucilaginous gum, which has many commercial outlets.

Table 2.1

Chemical composition of guar seed

(Per cent dry matter)

Constituent	Reference *				
	1	2	3	4	5
Crude protein	30.56	34.40	30.60	28.00	29.43
Ether extract	2.94	3.90	3.66	3.70	5.24
Crude fibre	10.00	5.20	9.81	5.00	10.52
Nitrogen-free extract	51.90	52.90	50.65	59.20	50.97
Ash	4.60	3.60	5.28	4.10	3.84
Calcium	0.29	0.35	0.35	0.27	0.29
Phosphorus	0.52	0.34	0.42	0.46	-

* 1 Srivastava and Singh (1960)

2 Singh et al. (1968)

3 Kawatra et al. (1968)

4 Patel et al. (1970)

5 Nagpal et al. (1971)

Table 2.2

Proportion and composition of guar seed fractions *

Per cent dry matter	Fractions			
	Seed coat	Endosperm	Cotyledons	Germ Embryo
Proportion of whole seed	17.11	38.50	42.78	1.61
Crude protein	9.90	8.30	65.60	62.10
Ether extract	0.70	1.70	6.90	12.00
Crude fibre	28.40	0.80	0.50	1.60
Nitrogen-free extract	57.00	88.60	21.80	20.20
Ash	4.00	0.60	5.20	4.10
Calcium	0.78	0.16	0.33	0.50
Phosphorus	0.05	0.03	0.70	0.83

* Source: Singh et al. (1968)

2.2 Chemistry and uses of guar gum

Guar gum, the refined endosperm found as a hard vitreous layer on the inner side of the seed coat, is separated from the rest of the seed components by a mechanical milling and sifting process. During the 1950's guar gum was the subject of much research in the United States where extensive studies on its chemical structure, properties and uses were carried out. Results of these experiments are described in detail by Goldstein and Alter (1959).

It is a neutral carbohydrate complex which on hydrolysis yields two hexoses, D-galactose and D-mannose in the ratio 1:2. The gum molecule is highly branched. Structural studies have shown that the polysaccharide consists of a backbone of D-mannopyranose units joined together by β-(1→4) linkages. Single D-galactopyranose units are joined to every alternate mannose residue by ∝-(1→6) linkages (Rafique and Smith, 1950). A representation of the structure for guar gum molecule proposed by Whistler and Smart (1953) is shown in figure 2.1.

The crude gum, which may contain 1-5% protein, is a greyish-white powder. At low concentrations in water it produces highly viscous solutions which possess stabilizing and emulsifying properties. Purified gum in very small amounts is used as a non-nutritive component for specific physicochemical purposes at 0.2 to 2.0% in a variety of food preparations such as frozen desserts, salad dressings, baked goods, cheese spreads etc. and at 0.0006 to 0.01% in beverages (Altman and Dittmer, 1968).

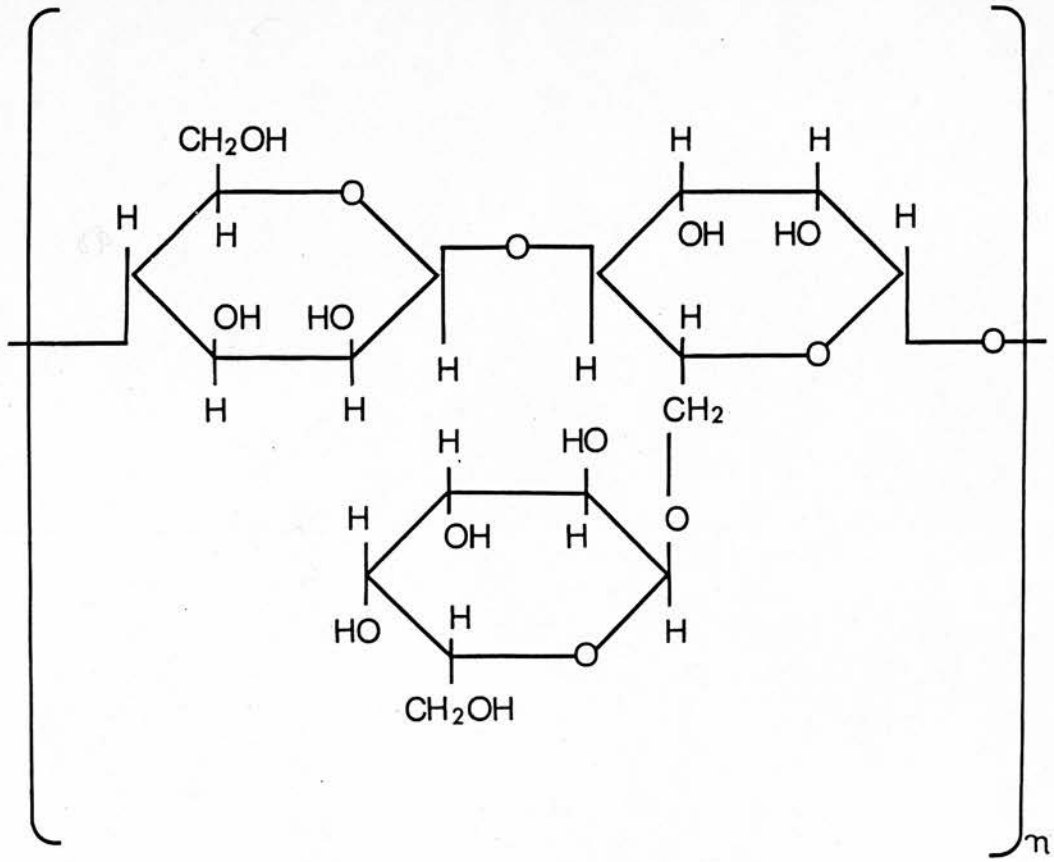


Fig. 2.1. Structure proposed for guar gum molecule.

source : Whistler and Smart (1953)

2.3 Composition of guar meal

Proximate composition

Data on the proximate composition of guar meal reported elsewhere and of the three samples used during this study are presented in tables 2.3 and 2.4 respectively. Because guar meal is obtained as a byproduct after separation from most of the endosperm, its chemical composition is dependent on the efficiency of the extraction process and might be expected to show considerable variation. Provided complete separation of the endosperm from the rest of the seed is achieved the product, mainly the germ and seed coat, theoretically should contain more than 50% crude protein. However, in practice this is difficult to achieve and the resultant products, guar gum and meal, are not entirely free from cross-contamination. This is reflected in the proximate composition of the several guar meal samples which have been analysed since 1960 (Table 2.3).

The main variations are seen in the amounts of crude protein, crude fibre and nitrogen-free extract, whereas the oil and ash contents remained relatively constant. The crude protein content of guar meal on a dry matter basis ranged from 38.78 to 46.92%, the lowest and highest values being reported by Nagpal et al. (1971) and Srivastava and Singh (1960) respectively. The three guar meal samples used during this study had similar crude protein contents with a mean value of 45.0 $\pm$ 0.3% on a dry matter basis, a value about 3 units higher than the mean value calculated from those reported elsewhere. About 88% of the crude protein determined in the three guar meal samples was found to be present as true protein.

Table 2.3

Proximate composition of guar meal from various sources

(Percent dry matter)

Constituent	Reference *							
	1	2	3	4	5	6	7	Mean SE
Dry matter	-	94.8	94.2	-	93.5	89.51	90.25	92.45 ± 1.07
Crude protein	46.92	41.10	42.70	43.57	39.50	42.87	38.78	42.21 ± 1.03
Ether extract	5.20	5.10	5.20	4.67	5.50	5.41	7.19	5.47 ± 0.30
Crude fibre	8.42	6.80	7.20	15.29	6.70	12.70	11.75	9.84 ± 1.29
Nitrogen-free extract	34.13	39.40	36.20	29.72	43.30	32.90	36.81	36.07 ± 1.67
Ash	5.33	7.60	8.70	6.75	5.00	6.12	5.47	6.41 ± 0.51

*
1 Srivastava and Singh (1960)
2-3 Shah et al. (1966)
4 Sadagopan and Talapatra (1968)
5 Patel et al. (1970)
6 Kawatra et al. (1968)
7 Nagpal et al. (1971)

Table 2.4

Chemical composition of guar meal samples used during this study

(% dry matter)

	Guar meal			
	GM-1	GM-2	GM-3	Mean SE
Dry matter	90.50	92.30	90.90	
Crude protein	44.40	45.18	45.39	45.0 ± 0.30
True protein	39.88	39.22	40.11	39.73 ± 0.26
Ether extract	5.54	5.74	5.89	5.72 ± 0.10
Crude fibre	10.22	9.91	10.12	10.08 ± 0.09
Available carbohydrates	16.35	15.49	16.83	16.22 ± 0.39
Total ash	6.83	5.68	5.37	5.96 ± 0.44
Acid-insoluble ash	1.48	0.64	0.14	0.75 ± 0.39

The values for diethyl ether-extractable oil contents of the various guar meal samples show very little variation. Except for one low value of 4.67% (Sadagopan and Talapatra, 1968) and a higher value of 7.19% (Nagpal et al., 1971) the oil content of the samples were found to be about 5.5% and are close to the mean value of 5.72 ± 0.1% obtained in the present study.

Mehta and Ramakrishnan (1957) studied the physicochemical properties of the oil extracted from guar seeds and stated it to be suitable for edible purposes. The contents of oleic, linoleic and linolenic acid were found to be 64, 19.1 and 0.4% respectively in addition to 16.4% saturated fatty acids. Although the oil contents of the meals are fairly low the presence of appreciable quantities of linoleic acid may be beneficial when the meal is included in diets for laying hens.

The ash contents of guar meal (table 2.3) range from 5.0 to 8.7% on a dry matter basis; the highest value being reported by Shah et al (1966). The three samples (table 2.4) used in this study were found to have 6.83, 5.68 and 5.37% ash respectively. The high ash contents in some of the guar meal samples (table 2.3) might be due to contamination of the material, e.g. by sand. The acid-insoluble ash contents of the samples used were measured and it was found that the content in GM-1 was about ten times that of GM-3.

The crude fibre contents of guar meal samples reported in the literature range from 6.7 to 15.29% of the moisture free material and showed the greatest variation. The mean crude fibre content of the three meals used in this study was found to be 10.08% (table 2.4). The high variations in the crude fibre contents presumably indicate both the limitations of the milling process

as well as of the methods used for crude fibre determination.

The nitrogen-free extract fraction is not determined directly but obtained by deducting from 100 the sum of the percentages of crude protein, crude fibre, diethyl ether extract and ash. Hence it cannot be regarded as a reliable index because it will be affected by the analytical errors associated with the determinations of the other nutrients. However it is customary to include the value in composition tables. The values for the nitrogen-free extract (NFE) of guar meal vary from 29.7 to 43.3%. The high variation associated with NFE values is also an indication of inconsistent milling and separation of guar seed endosperm. The amount of residual endosperm in the meal will probably have an inverse relationship with its protein content and this is apparent from the data in table 2.3.

Mineral composition

Data on the mineral constituents, other than calcium and phosphorus, in guar meal have not been widely reported. The three guar meal samples used in this investigation were, therefore, analysed for major and trace minerals. The results set out in table 2.5, show that there is good agreement amongst each set of values. The levels are normal for this type of feeding stuff, except that those for iron and potassium are high.

Amino acid composition

The total amino acid composition of a feed ingredient is required when formulating economic and nutritionally adequate diets for all catagories of poultry. The published amino acid composition of guar seed and guar meal and those of the three guar meal samples used in this study are set out in tables 2.6 and 2.7 respectively; the amino acid composition of soya bean meal is included for comparison.

Table 2.5

Mineral composition of guar meal samples used during this study

(on dry matter basis)

	GM-1	GM-2	GM-3
Calcium (%)	0.32	0.30	0.28
Phosphorus (%)	0.54	0.55	0.53
Magnesium (%)	0.24	0.26	0.26
Potassium (%)	1.60	1.68	1.52
Sodium (ppm)	77	70	71
Manganese (ppm)	19.6	19.3	18.2
Iron (ppm)	450	478	412
Zinc (ppm)	47.53	43.15	46.65
Copper (ppm)	11.25	11.75	11.30

Table 2.6

Amino acid composition of guar seed and guar meal as reported by different workers

(g/16g Nitrogen)

Reference/technique*	Guar seed			Guar meal		
	1	2	3	4	5	6
		cc	Ic	M		Ic
Alanine		0.23	4.2			
Arginine	2.3	2.05	12.5	10.19	8.89	15.91
Aspartic acid		10.20	10.2			
Glutamic acid		18.57	20.1			
Glycine		1.25	5.1		5.56	
Histidine	0.9	0.30	2.5	2.20	2.11	5.64
Isoleucine	3.7	4.20	3.2	3.45	3.11	
Leucine	6.1	16.10	5.9	4.56	6.22	
Lysine	5.2	10.30	4.0	4.40	4.11	6.95
Methionine	0.5	4.20	1.4	0.92	1.27	1.05
Cystine		0.27	0.6		1.40	
Phenylalanine	2.7	1.04	3.7	3.64	4.11	
Proline			3.1			
Serine		0.50	4.9			
Threonine	1.3	0.30	2.8	1.96	5.24	
Tryptophan	1.7	0.31	1.9	0.43	1.20	1.05
Tyrosine		0.70	3.3		4.58	
Valine	3.6	6.10	4.2	6.80	4.22	

*
cc Circular chromatography.
Ic Ion-exchange column chromatography.
M Microbiological assay
1 Block and Weiss (1956)
2 Ramakrishnan (1957)
3 Van Etten et al. (1961)
4 Ambegaokar (1969)
5 Titus and Fritz (1971)
6 Nagpal et al. (1971)

Considerable variability exists amongst the values reported by different workers for any individual amino acid (table 2.6). Differences in the analytical techniques employed, in addition to the possible differences in the materials used, may largely be responsible for the variations. Values for some amino acids show good agreement, others do not; for example, the high values for leucine, lysine and methionine, and the low values for histidine, serine, threonine, tryptophan and tyrosine reported by Ramakrishnan (1957) diverge from the rest. These values were obtained by circular paper chromatography over twenty years ago and the inaccuracies associated with the method offer the most obvious explanation for the differences. Furthermore the values of 5.24% for threonine (Titus and Fritz, 1971) and of 5.64% for histidine (Nagpal et al., 1971) appear to be high compared with those reported by other workers.

The three samples analysed and used in this laboratory are consistent in composition for most amino acids (table 2.6). Slight discrepancies, however, were noted in the methionine and tryptophan contents. GM-2 had an appreciably higher methionine level than the other meals whereas GM-1 had a lower value for tryptophan. The two sulphur-containing amino acids, methionine and cystine were determined after oxidation of the samples (Moore, 1963). In general guar meal protein appears to have a good distribution of essential amino acids; it is particularly rich in arginine but the level of sulphur-containing amino acids is low in relation to the requirement of poultry. This is characteristic of legumes.

Table 2.7

Amino acid contents of guar meal samples used during this study

(g/16g Nitrogen)

	GM-1	GM-2	GM-3	Soya bean meal [3]
Alanine	3.90	3.85	3.90	
Arginine	12.98	11.85	11.15	6.99
Aspartic acid	9.14	9.24	9.36	
Glutamic acid	16.27	15.02	18.23	
Glycine	5.23	5.02	4.99	5.87
Histidine	2.54	2.51	2.42	2.40
Isoleucine	3.28	3.18	3.05	5.74
Leucine	5.90	5.78	5.62	7.86
Lysine	4.20	4.00	4.17	6.20
Methionine*	0.85	1.44	0.95	1.44
½ cystine[1*]	1.26	1.39	1.36	1.46
Phenylalanine	3.58	3.71	3.55	4.80
Proline	2.74	2.44	2.80	
Serine	4.37	4.27	4.24	
Threonine	2.97	3.12	3.26	3.91
Tryptophan[2]	0.76	1.00	0.96	1.38
Tyrosine	4.54	4.24	4.17	3.06
Valine	3.72	3.12	3.57	5.24

* Determined after oxidation according to Moore (1963)
1 Cystine + cysteine expressed as ½ cystine
2 Determined by pronase method (Holz, 1972)
3 Titus and Fritz (1971)

Conclusions

Guar meal from its proximate composition, amino acid and mineral contents, appears to be an attractive product for use as a protein-rich ingredient in poultry feed formulations. However, its true nutritive value for poultry in terms of the availability and utilisation of various nutrients requires assessment biologically.

3. GUAR MEAL AS A FEED INGREDIENT - A REVIEW OF PUBLISHED REPORTS.

3.1 Ruminants

The ability of guar meal to spare commonly used ingredients in diets for the larger farm animals has been studied by a number of workers. Thus guar meal has been incorporated into diets for growing calves (Sadagopan and Talapatra, 1968), mature heifers (Srivastava and Singh, 1960), dairy cows (Thatte et al., 1967; Rahman and Leighton, 1968) and fattening cattle (Ala-ud-din et al., 1965) with no loss in performance. It has also been successfully used in place of cotton seed cake in diets for fattening sheep (Anwar et al., 1965; Malik et al., 1967).

Despite this success, however, it is generally acknowledged that guar meal is not liked by the animals when they are first presented with it in a diet, although thereafter they appear to acclimatise to it very quickly. This initial distaste has been attributed to its characteristic and quite pungent bean-like odour. In attempts to overcome this minor drawback, the effect of heating the meal on its palatability and, possibly, nutritive value has been investigated. In feeding trials with dairy cows neither Shah et al. (1966) nor Rahman and Leighton (1968) were able to observe any significant changes in the palatability or digestibility of diets containing guar meal as a result of toasting. Butler (1972) showed that toasted guar meal is a good substitute for soya bean meal in concentrate mixtures for heifers and Dijkstra (1962) found that toasted guar meal was not toxic when fed to wethers and had a digestibility coefficient of about 80%.

From these and other reports it is now accepted that guar meal, either raw or toasted, is a satisfactory food for the ruminant farm animal. Because toasting the meal does not completely free it from its characteristic odour and has little, if any, effect on its

nutritive value there seems no economic justification for heating before incorporating into a ruminant diet.

3.2 Monogastric animals

When guar beans or meal are fed to rats signs of toxicity appear almost immediately. These are: growth depression, reduced food intake and conversion efficiency, poorer protein utilisation and increased mortality (Borchers and Ackerson, 1950; Arrington et al., 1955; Kawatra et al., 1969a,b; Ambegaokar et al., 1969; Subramanian and Parpia, 1969). Subramanian and Parpia (1969) also showed that both raw guar meal and an isolated guar meal protein, when providing all of the dietary protein, were more toxic to weanling than to adult rats.

Efforts to utilize guar meal as a protein-rich ingredient in poultry diets started in the early nineteen sixties when supplies became commercially available. When included in diets of chicks guar meal has been reported adversely to affect the performance of chicks (Sathe and Bose, 1962; Ogra et al., 1963; Vogt and Penner, 1963; Vohra and Kratzer, 1964a and Bakshi et al., 1964); and of laying hens (Abeger, 1958; Fernandez and Santiago, 1961).

Vogt and Penner (1963) investigated the value of guar meal for broiler chicks from 0-8 weeks of age. When offered to the birds at dietary inclusion levels of 5, 10 and 15% it was found to depress food intake, growth rate and food conversion efficiency. In a similar study with female chicks growth depression and poor food conversion efficiency were observed; the depression increased with increasing dietary levels of guar meal (Keppens, 1964).

In attempts to overcome its growth depressing properties guar meal has been subjected to various pretreatment. Phillips

(1962) suggested the use of solvent extraction, and wet or dry heating to detoxify the meal. Bakshi et al (1964), and Vohra and Kratzer (1964a) observed that its nutritive value for the chick was improved by heating. Rao et al. (1966) investigated the effect on the performance of chicks from 0 to 4 weeks of age of substituting raw, alcohol-leached or water-soaked guar meal for ground nut meal at 6.5% dietary levels. It was claimed that there were no significant differences in the performance of chicks fed the different diets although birds fed diets containing guar meal in any form gained numerically less body weight and consumed less food than those fed the control diet.

Kawatra et al. (1968) separated guar meal into coarse, fine and germ-rich fractions and evaluated these before and after treatment, together with a commercially-toasted guar meal. The control diet was based on casein. The treatments were: soaking in 1% Na_2HPO_4 solution for 2 hr., cooking with water for 45 min., and soaking in water overnight. When fed to chicks from 0-6 weeks of age, the diets containing either the treated or the toasted guar meal were found to be much inferior in comparison with the control diet. Although none of the treatments was found to improve the meal, soaking overnight resulted in a lower rate of liveweight gain and an increase in mortality.

Couch et al. (1967) investigated the effect of heat treatment on the nutritional value of guar meal for broiler chicks. When 10% laboratory-processed guar meal (heating at 110°C for 60 min. followed by super-heated steam injection at 110°C for 15 min.) was substituted for soya bean meal the growth rates were the same. Similarly processed commercial guar meal was found to be satisfactory when included at 10% although higher rates of inclusion significantly

depressed growth. They suggested that the presence in the meal of some toxic factor, other than gum, was responsible for the growth depression.

Nagpal et al. (1971) examined the value of guar meal as a protein source in diets for chicks. The meal, which was found to have a lower gross protein value and metabolisable energy (ME) content than ground nut meal, was not improved by autoclaving for 30 min. at 15 psi. Inclusion of 40% guar meal in the diet of chickens resulted in negative nitrogen balance; and the ME of the diet was reduced.

Attempts to identify the toxic principle(s) in guar meal have been made for almost thirty years. In a study on the nutritive value of legume seeds, Borchers and Ackerson (1950) observed the growth-depressing effect of including guar seeds as the only source of protein in the diets of rats. This sample of guar which responded negatively to a trypsin-inhibitor test, was not improved by autoclaving for 30 min. at 15 psi. However Ambegaokar et al. (1969), who also noted a growth depressing effect of feeding diets containing 42% guar meal to rats, observed a beneficial effect after autoclaving the meal. The effect was enhanced by 1% supplemental methionine.

Kawatra et al. (1969a) attempted to detoxify the guar meal by extracting it with boiling water, treating it with IN HCl or supplementing it with enzymes isolated from sprouted guar seeds. When fed to provide 10% protein in diets for rats the hot water-extracted and acid-treated meals considerably improved the growth rate; the enzyme treatment however, did not appear to do so. The acid-and hot water-treatments of the meal resulted in yields of 60 and 70% respectively.

In another report Kawatra et al. (1969b) described the beneficial effect that supplemental methionine and lysine had on the utilisation of diets containing hot water- and HCl-treated guar meals by rats. When raw guar meal with and without the addition of methionine and lysine supplied 10% of the protein in diets of rats mortality was 100 and 86% respectively.

Couch et al. (1966) provided evidence for the presence of a toxic factor, which was tentatively judged to be a trypsin inhibitor, in a guar meal extract prepared in phosphate buffer (pH 7.6, 0.02M). Heating the meal or this extract for 60 min. destroyed 80% of the activity. The inhibitor, which was non-dializable, was probably a macromolecule.

In the same laboratory Hooper and Couch (1971) isolated a toxic factor from guar and identified it as a trypsin inhibitor. The factor was reported to be a protein. It was stable over a pH range of 2-11 and reacted with trypsin in a 1:1 molar ratio.

Earlier Tannous and Ullah (1969) had failed to demonstrate any antitryptic activity in guar beans (seeds) although the seeds were toxic when fed to rats. However, they found guar beans possessed some haemagglutinating activity against chicken blood cells. The activity was of a very low order, 20 units as compared with 10,560 units found in kidney beans (Phaseolus vulgaris L.); 8,200 units in common beans (Phaseolus vulgaris L.) and 80 units in broad beans (Vicia faba L.). Autoclaving guar beans for 5 min. at 121^{o}C was found effectively to destroy all the haemagglutinating activity. Guar beans were not found to possess haemagglutinating activity against rabbit, rat or human blood according to Huprikar and Sohonie (1960).

In addition to the trypsin inhibitor and haemagglutinin present in the meal, the growth depression caused by its inclusion in the diet of chicks has also been attributed to the presence of residual gum. Support for this view comes from the observation that droppings from animals fed on diets containing guar meal tend to be sticky.

Vohra and Kratzer (1964a,b) studied the effect of ingestion of guar meal and certain polysaccharides by chicks and noted that inclusion of as low as 0.25% guar gum in their diet depressed growth rate although feed conversion efficiency was little affected. Increasing the dietary level of gum to 2% caused a 25-30% growth depression which could be overcome to a large extent by treatment with an isolated guar enzyme or a commercial cellulase preparation. In a further study they (Vohra and Kratzer, 1965) observed a marked improvement in the growth of chicks when their diets, which contained about 20% toasted or autoclaved guar meal were supplemented with enzymes (cellulases).

At the same time experiments with chicks, based on previous experience with certain enzymes in improving the value of diets containing barley, were carried out by Anderson and Warnick (1964) to evaluate a number of enzyme preparations in diets containing guar gum or meal. Although certain enzyme preparations were found effective in counteracting the adverse effects produced by guar gum or meal on chicks, addition of those enzymes which had been found beneficial in diets containing barley did not improve the growth rate or feed efficiency of chicks fed diets containing guar meal. These findings point to a difference in the structure of barley and guar gums.

Rats appear able to tolerate much higher amounts of guar gum in their diets than do chicks (Ershoff and Wells, 1962); no significant depression in growth was found even when they fed on a diet containing as much as 10% gum. Likewise Booth *et al*. (1963) observed no significant growth depression in rats fed on diets containing 6% guar gum and reported that this polysaccharide was 76% digestible. However the utilisation of the gum based on weight gain by rats was very low and therefore the digestibility value should possibly be treated with caution. These reports suggest that guar meal may vary widely in their undesirable effects on monogastric animals.

3.3 Conclusions and projection for this study

This survey of the published reports indicates that the nutritional value of guar meal for poultry is not fully understood. Furthermore the effects of its inclusion in poultry feed formulations have neither been examined in large scale feeding trials nor have there been many attempts to evaluate this by-product for its metabolisable energy value and protein quality.

In view of the ever -increasing demand for food and the growing competition between man and monogastric animals, particularly poultry, for conventional feed ingredients, the search for newer food sources or the effective utilisation of those available in animal feeding is becoming more and more urgent.

Because guar meal is available in appreciable quantities and because of the problems that have been encountered when it is included in poultry diets, a detailed investigation of its value to poultry was considered to be a matter of the utmost importance. In order that its potential nutritional value for poultry can be better understood,

guar meal:

a) was examined for its chemical components,

b) included in broiler feed formulations,

c) included in the feed formulations of laying hens,

d) bio-assayed for its protein quality,

e) evaluated for its metabolisable energy content, and

f) efforts were made to enhance its nutritive value for poultry by processing, extraction and supplementation with amino acids and enzymes.

4. BROILER GROWTH

4.1 Broiler experiment 1

Object

The experiment was conducted to measure the response of broiler chickens when a sample of commercial guar meal was included in their diets as a protein source. The effects of heating the meal and of supplementation of diets containing the meal with methionine on the performance of the birds were also studied.

Experimental design

Forty-eight groups, each of about 40 male or 40 female day-old broiler chicks, were randomly assigned to receive one of eight experimental diets from 0-8 weeks of age. The experiment was of a 8 x 2 x 3 factorial design where the factors were dietary treatment, sex and block respectively.

Birds and management

About nineteen hundred Ross I broiler chicks were floor-brooded in 48 pens under one roof and in a controlled environment where the air and brooding temperatures and ventilation rate followed the recommendations of the breeder. The pens had a layer of wood shavings on the floor. Infra-red brooding lamps were provided for the first 7 day period after which the lamps and the brooder surrounds were removed. A floor area of about 0.09 m^2 per bird was allowed. Male and female chicks were allocated randomly, at 1 day of age, to the separate pens where food and drinking water were available ad libitum at all times. Birds in each pen were weighed at 4 and 8 weeks of age. Food eaten per pen was recorded weekly. Mortality was also recorded.

Heat treatment of guar meal

Guar meal was spread out evenly on metal trays to a depth of about 16mm and placed in a forced-draft hot-air oven at 107^{o}C for 60 min. A second batch of guar meal was similarly heated for 120 min.

Immediately after heating, the meal was removed from the oven and transferred to other trays where it was spread in thin layers and left overnight to cool and to recover part of moisture lost during heating. The commercial sample of guar meal used in this experiment was referred to as GM-1.

Diets

The composition of the eight starter and eight finisher diets fed during 0-28d and 29-56d of age are set out in tables 4.1 and 4.2 respectively. Diet 1A, which acted as control, was formulated to supply all the chicks nutrient requirements (NRC, 1971). Diet 1B was similar to 1A except that 4 parts of soya bean meal and 1 part of wheat were replaced by 5 parts of commercial guar meal. Diets 1C, 1D and 1E were also similar to 1A except that 8 parts of soya bean meal and 2 parts of wheat were replaced by 10 parts of commercial guar meal, of guar meal which had been heated for 1 hour and of guar meal heated for 2 hours respectively. Diets 1F, 1G and 1H were similar to 1C, 1D and 1E respectively except that they were all supplemented with 0.5% DL-methionine. All diets were calculated to be isonitrogenous and isocaloric. The composition of each starter diet was slightly modified to obtain the composition of the corresponding finisher diet to take into account the different energy and protein requirements of the chicks after four weeks of age. The diets were offered as pellets of about 3mm diameter from 0-4 weeks and of about 5mm from 5 to 8 weeks of age. All diets were analysed for moisture, crude protein, ether extract, total ash, calcium and phosphorus, the results of which together with the calculated methionine and metabolisable energy contents are set out in Appendix B5.

Table 4.1

Composition of starter diets

Ingredients (g/kg of diet)	Diet 1A	1B	1C	1D	1E	1F	1G	1H
Maize	307	302	297	297	297	292	292	292
Wheat	230	220	210	210	210	205	205	205
Soya bean meal	260	220	180	180	180	180	180	180
Guar meal (GM-1)	-	50	100	-	-	100	-	-
Guar meal, heated 1 hour	-	-	-	100	-	-	100	-
Guar meal, heated 2 hours	-	-	-	-	100	-	-	100
Maize oil	24.5	29.5	34.5	34.5	34.5	39.5	39.5	39.5
DL-Methionine	2	2	2	2	2	7	7	7
Supplements*	176.5	176.5	176.5	176.5	176.5	176.5	176.5	176.5

* Meat-and-bone meal, 110; maize germ meal, 50; $CaCO_3$, 4; $CaHPO_4$, 5; choline chloride, 0.5; NaCl, 2; vitamin premix**, 2.5; mineral premix**, 2.5.

** Compositions of vitamin and mineral premixes are set out in Appendix B1.

Table 4.2

Composition of finisher diets.

Ingredients (g/kg of diet)	Diet 1A	1B	1C	1D	1E	1F	1G	1H
Maize	307	302	297	297	297	292	292	292
Wheat	300	290	280	280	280	275	275	275
Soya bean meal	210	170	130	130	130	130	130	130
Guar meal (GM-1)	-	50	100	-	-	100	-	-
Guar meal, heated 1 hour	-	-	-	100	-	-	100	-
Guar meal, heated 2 hours	-	-	-	-	100	-	-	100
Maize oil	24.5	29.5	34.5	34.5	34.5	39.5	39.5	39.5
DL-Methionine	2	2	2	2	2	7	7	7
Supplements*	156.5	156.5	156.5	156.5	156.5	156.5	156.5	156.5

* Meat-and-bone meal, 90; maize germ meal, 50; $CaCO_3$, 4; $CaHPO_4$,5; Choline chloride, 0.5; NaCl, 2; Vitamin premix**, 2.5; Mineral premix**, 2.5.

** Compositions of vitamin and mineral premixes are set out in Appendix B1.

In Tables 4.3 to 4.5 the Standard Errors were calculated from the Mean Square Error in the appropriate Analysis of Variance Table (28 degrees of freedom) using the 6 replicates on each treatment. The probability values quoted refer to the variance ratio test for overall treatment differences which has an F-distribution with 7, 28 degrees of freedom

Results

The performance of chicks has been considered in three periods, 0-28d, 0-56d, and 29-56d of age. In addition to the effects of the level of dietary guar meal on performance the data were examined for any effects caused by heat treatment and by methionine supplementation.

0-28d period

The liveweights, food intakes and food conversion efficiencies (FCE) of male and female chicks fed on the different diets are summarised in table 4.3. It can be seen that chicks, both male and female, fed on the control diet (1A) weighed significantly more ($P < 0.01$) than those fed on diets containing guar meal (1B to 1H). Also chicks fed on the diets containing 5% guar meal (1B) performed significantly better ($P < 0.01$) than those fed on diets containing 10% guar meal.

0-56d period

Data on growth, food intakes and FCE of male and female chicks for the entire 8 week period of the experiment are summarised in table 4.4. Chicks fed on the control diet (1A) performed significantly better ($P < 0.01$) than those fed diets containing 10% guar meal (1C to 1H). Male birds in all the dietary groups consistently weighed more and consumed more food than the female chicks; although males were generally more efficient there was little difference between the FCE of the sexes. At 8 weeks of age male birds fed on the diet containing 5% guar meal had almost the same body weights as control-fed birds but, because they had eaten significantly more food ($P < 0.01$), had poorer FCE values.

Table 4.3

Performance of broilers from 0 to 28d of age

	Diet									
	1A	1B	1C	1D	1E	1F	1G	1H	Mean	
Mean live-weight at 28d (g)										
Male	835	726	582	517	525	523	504	492	588	SE ± 8.8**
Female	765	642	516	484	503	455	393	427	523	
Mean	800	684	549	501	514	489	448	460		
				SE ± 17.5***						
Mean food intake (g)										
Male	1301	1190	1056	1005	981	934	920	901	1306	SE ± 8.3**
Female	1173	1069	938	891	900	821	846	841	936	
Mean	1237	1130	997	948	940	877	883	871		
				SE ± 16.7***						
Food conversion efficiency (live-weight, g/100g food consumed)										
Male	61.81	63.45	52.80	53.85	51.27	58.20	52.70	56.90	56.37	SE ± 0.73NS
Female	67.53	57.75	57.33	52.36	58.22	53.05	49.01	48.51	55.47	
Mean	64.67	60.60	55.07	53.11	54.74	55.63	50.85	52.70		
				SE ± 1.46***						

NS = not significant; ** = P < 0.01; *** P = < 0.001

Table 4.4

Performance of broilers from 0 to 56d of age

	Diet									
	1A	1B	1C	1D	1E	1F	1G	1H	Mean	
Mean live-weight at 56d (g)										
Male	2146	1998	1761	1631	1701	1582	1698	1539	1757	SE
Female	1711	1725	1372	1401	1383	1380	1289	1389	1456	± 13 ***
Mean	1928	1862	1567	1516	1542	1481	1494	1464		
					SE ± 26 ***					
Mean food intake (g)										
Male	4866	4589	4386	4011	4228	3813	4084	3656	4204	SE
Female	3916	4203	3480	3532	3389	3464	3042	3396	3553	± 23 ***
Mean	4391	4396	3933	3772	3809	3639	3563	3526		
					SE ± 46 ***					
Food conversion efficiency (live-weight, g/100g food consumed)										
Male	44.10	44.99	38.62	42.19	38.73	42.94	40.14	43.51	41.72	SE
Female	43.69	39.58	40.96	38.16	42.24	38.44	43.66	39.54	40.95	± 0.19*
Mean	43.85	42.29	39.79	40.18	40.48	40.69	41.90	41.53		
					SE ± 0.38 ***					

* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

29-56d period

Data on weight gains, food intakes and FCE of male and female chicks during the last four weeks of the experimental period are presented in table 4.5. It can be seen that chicks fed on diets containing 10% guar meal gained significantly less body weight ($P < 0.01$) and also consumed significantly less food than the controls. Male chicks in all the groups consistently weighed more and consumed more food than female chicks; female chicks had the poorer FCE values except in dietary groups 1G and 1H where they had better FCE ($P < 0.05$) than the corresponding male chicks. No significant differences ($P < 0.05$) were noticed amongst the FCE of chicks (combined sexes) fed diets 1A to 1F, whereas those fed on diets containing heated guar meal for 1 and 2 hours respectively plus added methionine (1G and 1H) had better FCE than the chicks fed on the control diet (1A) or unsupplemented diets containing guar meal (1B to 1F).

Effect of heat treatment

The effect of heating guar meal on the performance indices of the chicks is summarised in table 4.6. Toasting the meal at 107^{o}C for 1 or 2 hours did not appreciably influence the performance of the chicks when fed at the 10% dietary level. Chicks fed on diets containing toasted guar meal (1D, 1E, 1G and 1H) consumed less food than those fed on diets containing commercial guar meal (1C, 1F), the effect being significant during the 29-56d period and with female chicks ($P < 0.05$). No signficant differences on the body weights of chicks fed on different diets were noticed.

Table 4.5

Performance of broilers from 29 to 56d of age

	Diet									
	1A	1B	1C	1D	1E	1F	1G	1H	Mean	
Mean gain in body weight (g)										
Male	1311	1272	1179	1114	1177	1059	1195	1048	1169	SE ± 14***
Female	945	1084	856	916	880	926	896	962	933	
Mean	1128	1178	1017	1015	1028	993	1045	1005		
				SE ± 28 ***						
Mean food intake (g)										
Male	3527	3438	3291	3045	3208	2918	3125	2795	3168	SE ± 23***
Female	2782	3095	2582	2602	2529	2605	2235	2516	2618	
Mean	3154	3266	2936	2823	2868	2761	2680	2655		
				SE ± 46 ***						
Food conversion efficiency (weight-gain, g/100g food consumed)										
Male	37.28	36.80	35.91	36.48	36.80	36.18	38.45	37.36	36.91	SE ±
Female	33.96	35.05	33.28	35.07	34.79	35.63	39.80	38.37	35.74	0.38 *
Mean	35.62	35.92	34.59	35.78	35.80	35.90	39.12	37.87		
				SE ± 0.76 **						

Table 4.6

Effect of heat treatment of guar meal on the performance of broiler chicks

Mean of diets	0-28d			0-56d			29-56d		
	Live wt.	Food intake	FCE	Live wt.	Food intake	FCE	Weight gain	Food intake	FCE
Male chicks									
1C,1F	552	995	55.5	1671.6	4099	40.8	1119.1	3105	36.0
1D,1G	510	962	53.3	1664.4	4042	41.2	1154.4	3085	37.5
1E,1H	508	941	54.0	1620.3	3942	41.1	1112.3	3001	37.1
SE	17.5	17	1.5	26.1	53.3	0.38	28.4	46	0.76
Female chicks									
1C,1F	485	879	55.2	1376.2	3472	39.7	890.8	2593	34.5
1D,1G	439	869	50.7	1344.6	3287	40.9	905.7	2418	37.4
1E,1H	465	871	53.4	1385.9	3393	40.9	920.7	2522	36.6
SE	17.5	17	1.5*	26.1	53.3*	0.38	28.4	46*	0.76*
Combined sexes									
1C,1F	519	936	55.3	1523.9	3785	40.3	1004.9	2849	35.3
1D,1G	474	916	52.0	1504.5	3667	41.1	1030.1	2751	37.5
1E,1H	487	906	53.7	1503.1	3667	41.0	1016.5	2761	36.9
SE	12.4	11.7	1.0*	18.4	38*	0.27	20.7	32*	0.5*

* = $P < 0.05$

Effect of dietary methionine supplementation

The effect of methionine supplementation of diets containing 10% guar meal on the performance of chicks was examined and the results are summarised in Table 4.7

Birds fed on diets containing 10% guar meal with 0.5% supplemental methionine (1F, 1G and 1H) weighed significantly less ($P < 0.01$) at 28d of age than those fed on the corresponding unsupplemented diets (1C, 1D and 1E). Because the food intake of chicks in these two groups also differed, no difference in FCE of chicks between the two groups was noted when data for the combined sexes was analysed. However female chicks fed on methionine-supplemented diets (1F to 1H) had lower FCE ($P < 0.05$) than females fed on unsupplemented diets (1C to 1E).

During the latter part (29-56d) of the growth period no significant differences were detected in the weight gains of chicks fed on 10% guar meal diets with or without added methionine. The food intake of chicks fed on diets 1F, 1G and 1H was, however, lower ($P < 0.001$) than those fed on diets 1C, 1D and 1E so that chicks receiving additional methionine in the diet had significantly better FCE ($P < 0.001$). The response of female chicks to supplemental methionine during this period of growth was greater than that of males.

Consideration of the overall performance (0-56d) showed that chicks fed on diets with added methionine (1F, 1G, and 1H) were significantly lighter in weight ($P < 0.05$). Despite consuming much less food ($P < 0.001$) than those fed on diets 1C, 1D, and 1E these birds still showed significantly better FCE ($P < 0.05$). Although chicks of both sexes had better FCE when fed on diets containing added methionine, males showed the greater response.

Table 4.7

Effect of methionine supplementation in diets containing 10% guar meal on the performance of broiler chicks

Male chicks

Mean of diets	0-28d Live wt	0-28d Food intake	0-28d FCE	0-56d Live wt	0-56d Food intake	0-56d FCE	29-56d Weight gain	29-56d Food intake	29-56d FCE
1C,1D,1E	541.1	1014	52.6	1697.6	4208	39.9	1156.6	3181	36.4
1F,1G,1H	506.0	918	55.9	1606.5	3851	42.2	1100.6	2946	37.3
SED between means	20.2	19.2***	1.7	30.1*	62***	0.4***	32.8	53***	0.9

Female chicks

1C,1D,1E	501.9	910	56.0	1385.2	3467	40.5	883.9	2572	34.4
1F,1G,1H	425.0	836	50.2	1352.6	3301	40.5	927.6	3452	37.9
SED between means	20.2***	19.2***	1.7*	30.1	62	0.4	32.8	53	0.9***

Combined sexes

1C,1D,1E	521.2	962	54.3	1541.5	3837	40.2	1020.3	2876	35.4
1F,1G,1H	465.5	877	53.1	1479.6	3576	41.3	1014.1	2699	37.6
SED between means	14.3***	13.6***	1.2	21.3*	43.6***	0.3*	23.2	37***	0.6***

* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

Mortality

The overall mortality in 0-56d period was 2.7%. Approximately 80% of the total mortality took place during the initial 4 week period and most of that during the first ten days. No consistent differences in mortality due to the dietary treatments or between the male and female birds was observed.

4.2 Broiler experiment 2

Object

This experiment was designed with the following objectives -

1) to study the effect of the dietary inclusion of a second sample of guar meal (GM-2) on the performance of broilers,

2) to study the effect of toasting and autoclaving the meal on its nutritive value,

3) to study the effect of supplementation with higher doses of vitamins and of methionine in diets containing 15% guar meal on the performance of chicks.

Experimental design

The experiment was a 4 x 4 x 3 factorial in a completely randomised design, where the factors were: dietary treatments (guar meal), vitamins and methionine levels and replicates respectively. There were sixteen diets, each of which was randomly assigned to three pens of six chicks and fed as mash for a two-week period when the birds were between 1 to 3 weeks of age.

Birds and management

About 330, Ross I male broiler chicks from the same hatch were wing-banded and reared to one week of age in thermostatically controlled battery brooders. During the rearing period they were fed on a broiler starter mash (Appendix B4). On the 8th day all chicks were individually weighed to the nearest gramme; 288 chicks were selected from the middle band and distributed to one of 48 cages, according to a previously randomised plan, so that each cage contained six chicks. The cages were fitted with food and water

troughs and raised wire floors. They were all located in the same room with the environment controlled. Food and drinking water were available at all times. Individual body weights of the birds and food intake for each pen were recorded at weekly intervals.

Heat treatment of guar meal

The guar meal, GM-2, used in this experiment was a commercial sample. In the absence of any definite details regarding its pretreatment it was decided to process it in the following ways.

Toasting

Guar meal was subjected to dry heat in the same way as described for experiment 1. The material was spread out evenly to a depth of about 16mm on metal trays and heated in a forced-draft hot-air oven at 107°C for 60 min. Following heat treatment the material was left overnight at room temperature both to cool and to regain part of the moisture lost during the heating process.

Autoclaving

Guar meal was spread out in layers of about 16mm deep on to aluminium foil-lined metal trays and autoclaved at 121°C for 30 min. To prevent condensed water falling on top of the material the trays were covered with aluminium foil. After autoclaving the meal was transferred to another tray and allowed to cool and dry before incorporating in diets.

Diets

There were four basal diets, the compositions of which are set out in Table 4.8. Diet 2A was formulated to supply the chicks' requirement for known nutrients (NRC, 1971) and served as a control. Diets 2B, 2C, and 2D were similar to diet 2A except that they contained 150g guar meal, toasted guar meal or autoclaved guar meal per kg respectively, substituted mainly for soya bean meal. All the diets

Table 4.8

Composition of diets (g/kg)

	Diet			
	2A	2B	2C	2D
Maize	307	292	292	292
Wheat	230	200	200	200
Soya bean meal	260	140	140	140
Guar meal (GM-2)	-	150	-	-
GM-2, heated	-	-	150	-
GM-2, autoclaved	-	-	-	150
Maize oil	24.5	39.5	39.5	39.5
Supplements*	178.5	178.5	178.5	178.5
Analyses				
Crude protein (N X 6.25)	221	222	225	226
Calcium	11	11	11	11
Phosphorus	7	7	7	7
ME (kcal/kg, calculated)	3000	3000	3000	3000

* Meat-and-bone meal, 110; maize germ meal, 50; $CaCO_3$, 4; $CaHPO_4$, 5; choline chloride, 0.5; NaCl, 2; methionine, 2; vitamin premix**, 2.5; mineral premix**, 2.5.

** Compositions of vitamin and mineral premixes are given in Appendix B1.

were made isocaloric and isonitrogenous by making slight adjustments to their wheat and oil contents.

In addition to the four basal diets another twelve diets were mixed. These diets were obtained by supplementing each of the four basal diets with twice normal (2N), thrice normal (3N) amounts of vitamin premix (Appendix B1), and thrice normal amounts of vitamin premix plus 5g methionine (3N + M) per kg respectively. Each of the sixteen diets were offered for two weeks to triplicate lots of six chicks.

Results

The results from this experiment are summaried in table 4.9. Data were analysed statistically by analysis of variance the table of which is contained in Appendix C1.

Weight-gain

Chicks fed on the control diet (2A) gained significantly more weight ($P < 0.001$) than those fed on diets containing 15% guar meal, toasted guar meal or autoclaved guar meal (2B, 2C and 2D) respectively. Addition of extra vitamins or of extra vitamins and methionine to the control diet did not improve the growth rates of chicks. Birds fed on diets 2B and 2C, containing commercial and toasted guar meal respectively, did not differ in their growth rates, whereas autoclaving the meal probably improved the growth of chicks ($P < 0.05$) over those fed on diets containing the commercial or the toasted guar meal. Increasing the level of vitamins from normal to three times normal and adding 0.5% methionine to diets containing 15% commercial, toasted or autoclaved guar meal did not influence the weight gains by chicks fed on them under the conditions of this experiment. There were no significant interactions.

Table 4.9

Performance of broilers fed diets containing differently treated guar meals, additional vitamins and methionine

Vitamin level & methionine	2A	2B	2C	2D	Mean	
Mean weight-gain (g), 8-21d of age						
1N	370	279	298	302	312	
2N	360	295	291	310	314	SED ±
3N	363	284	276	296	305	6.58
3N + M	372	296	279	309	314	
Mean	366	289	286	304		
			SED ± 6.58***			

SE of difference between means in body of table ± 16.12

Mean food intake (g)						
1N	575	560	563	543	560	
2N	592	575	539	564	568	SED ±
3N	628	584	562	556	582	12.13
3N + M	603	535	504	565	552	
Mean	599	564	542	557		
			SED ± 12.13***			

SE of difference between means in body of table ± 29.72

Food conversion efficiency (Weight-gain, g/100g food consumed)						
1N	64.63	49.67	52.96	55.70	55.74	
2N	60.85	51.28	54.29	54.89	55.33	SED ±
3N	57.80	48.79	49.20	53.16	52.25	1.37*
3N + M	61.73	55.39	55.49	54.74	56.84	
Mean	61.25	51.28	52.98	54.62		
			SED ± 1.37***			

SE of difference between means in body of table ± 3.36

* = $P < 0.05$; *** = $P < 0.001$.

Food consumption

Birds fed on the control diet (2A) consumed significantly ($P < 0.001$) more food than those fed on diets containing guar meal. Although no significant differences existed in the food intakes of chicks fed on diets 2B, 2C or 2D, containing variously treated guar meals, food consumption was numerically lowest on the diet containing toasted meal.

Increasing the level of vitamins in the control or guar meal-containing diets from normal to thrice normal levels appeared to be associated with an increased, though not significant, food intakes by birds. Addition of 0.5% methionine in diets probably decreased, though again not significantly, the food intakes of chicks. There were no signficant interactions.

Food conversion efficiency

Birds given the control diet (2A) recorded the best FCE and those fed on the diet containing commercial guar meal (2B) the poorest. No significant difference between the FCE of chicks fed on diets containing commercial or toasted guar meal was found. The diet containing the autoclaved guar meal (2D) was probably utilised better ($P < 0.05$) than that containing commercial guar meal (2B).

Increasing the vitamin level from normal to thrice normal, in the control as well as in the guar meal-containing diets, appeared to have an adverse effect on the FCE of chicks ($P < 0.05$). This effect was reversed when diets were supplemented with 0.5% methionine. There were no significant interactions.

Mortality

There was no mortality during the course of the experiment.

4.3 Broiler experiment 3

Object

In a previous experiment (4.1) diets containing guar meal, before or after heat treatment, were fed to broilers from 0-56d of age. It was observed that heating did not improve the nutritive value of the meal. The birds, however, appeared to utilise the diets containing guar meal more efficiently during the 5-8 week period than during 0 to 4 weeks of age.

One of the factors in guar meal considered deleterious to chick performance is the residual gum. Certain commercial enzyme preparations are known to hydrolyse similar gums and mucilages and it was decided to study the efficacy of two such preparations on the utilisation of the meal.

Steam pelleting is known to influence the nutritive value of certain feeds and feed ingredients. Its effects on diets containing guar meal was also considered worthy of investigation.

The object of the experiment, therefore, was to study the effect on chicks from 29 to 56 days of diets containing guar meal (GM-3) offered as mash or pellets, and of supplementing them with one of two enzyme preparations.

Experimental design

Forty-eight groups, each of about 40 male 4 week old broiler chicks were randomly assigned to receive one of twelve diets, either as mash or pellets, from 29 to 56d of age. The experiment was of a 4 x 3 x 2 x 2 factorial design where factors were level of guar meal, enzyme, dietary form and block respectively.

Birds and management

About two thousand, one-day-old, Ross I male broiler chicks

were randomly allotted to 48 pens and floor brooded in a controlled-environment house as described earlier (4.1.). From day-old to 28-d of age chicks were fed a common broiler starter diet (Diet 1A, table 4.1), half in mash form and another half in pellet form. Food and drinking water were available to birds at all times. They were weighed at 4 and 8 weeks of age. Food intakes per pen were recorded weekly. Any birds dying during the experimental period were recorded.

On day 29 the experimental diets were allocated to the groups according to a randomised plan, except that the form of the diet was not changed.

Diets

The composition of the four basal diets are set out in table 4.10. A third consignment of guar meal (GM-3) was substituted at 0, 5, 10 and 15% dietary levels mainly for soya bean meal in the control diet. Dietary levels of wheat and maize oil were slightly modified to maintain them isocaloric and isonitrogenous by calculation. All diets were analysed for their proximate components and found to confirm the calculated values.

Two enzyme preparations, MKC hemicellulase[1] and Betaganase[2] M were added separately at 0.05 and 0.01% dietary levels respectively to each of the four basal diets, thereby raising the number of experimental diets to twelve. Each of the twelve diets was fed separately in two forms, mash and pellets (5 mm), to duplicate lots of chicks from 29-56d age. The dietary treatments were allocated within two blocks according to a randomised plan prepared beforehand.

1. A product of Miles Kali-chemie GmbH & Co
2. Supplied by Powell & Scholefield Ltd., Liverpool, England.

Table 4.10

Composition of four basal experimental diets fed to broilers from 29-56d of age

Ingredients (g/kg of diet)	Diet 3A	3B	3C	3D
Maize	316	316	316	316
Wheat	300	282	264	246
Soya bean meal	200	160	120	80
Guar meal (GM-3)	-	50	100	150
Maize oil	24.5	32.5	40.5	48.5
Supplements*	159.5	159.5	159.5	159.5
Calculated analysis				
Crude protein	200	200	200	200
Calcium	11	11	11	11
Phosphorus	7	7	7	7
ME (kcal/kg)	2970	2970	2980	2985

* Meat-and-bone meal, 90; maize germ meal, 50; $CaCO_3$, 4; $CaHPO_4$, 5; choline chloride, 0.5; methionine, 2; NaCl, 2; Lysine, 1; vitamin premix**, 2.5; mineral premix**, 2.5.

** Compositions of vitamin and mineral premixes are set out in Appendix B1.

Body composition

At the end of the experiment two chicks from each pen (4 chicks per dietary treatment) were taken at random for analysis of body composition. The birds were killed by cervical dislocation and frozen. Each frozen carcass was chopped and minced to a homogenous paste. Duplicate samples, each of about 100g, were transferred to aluminium dishes, and freeze-dried. The loss in weight was recorded as moisture. Nitrogen, oil and total mineral contents of the dried samples were determined in duplicate on each sample using standard methods of analyses (AOAC., 1965).

Results

The mean live-weight gains of chicks, food intakes and food conversion efficiencies during the experimental period of four weeks are summarized in tables 4.11 and 4.12. The results of the analysis of variance are set out in Appendix C2. Allowance was made for differences in the 4-week weights of chicks by analysis of covariance in order to have better assessments of the responses to the various dietary treatments during 29-56d period.

Weight-gain

Chicks fed on diets containing 10 and 15% guar meal (3C and 3D) gained significantly less weight ($P < 0.001$) than those fed on the control diet (3A). As little as 5% inclusion of guar meal in the diet (3B) appeared to depress growth though not significantly. The addition of MKC hemicellulase or Betaganase M to diets containing 5, 10, or 15% guar meal (3B to 3D) improved the growth of chicks significantly ($P < 0.01$) compared with those fed the corresponding diets with no added enzymes, but did not significantly improve the growth of chicks fed on the control diet. At the levels tested, although they did not differ significantly in their beneficial

Table 4.11

Performance of broilers fed diets containing guar meal from 29 to 56d of age

	Diet					
	3A	3B	3C	3D	Mean	
Mean weight-gain (g), 29-56d of age						
E0	1384	1330	1198	1118	1258	
E1	1389	1410	1337	1259	1349	SE ± 12.32***
E2	1406	1396	1330	1227	1340	
Mean	1393	1378	1288	1201		
			SE ± 13.15***			
			SE of the mean in body of the table ± 23.17			
Mean food intake (g), 29-56d of age						
E0	3526	2576	3444	3389	3484	
E1	3606	3676	3598	3554	3609	SE ± 23.7 **
E2	3606	3628	3540	3472	3561	
Mean	3579	3626	3527	3472		
			SE ± 27.8 **			
			SE of the mean in body of the table ± 48.2			
Food conversion efficiency (Weight gain, g/100 g food)						
E0	39.03	37.22	34.68	32.98	35.98	
E1	38.63	38.30	37.42	35.47	37.46	SE ± 0.23***
E2	39.11	38.38	37.45	35.36	37.57	
Mean	38.92	37.97	36.52	34.60		
			SE ± 0.25***			
			SE of the mean in body of the table ± 0.458			

E0 = No added enzyme; E1 = MKC Hemicellulase; E2 = Betaganase M

** = $P < 0.01$; *** = $P < 0.001$

Table 4.12

Effect of dietary form on the performance of chicks

	Diet					
	3A	3B	3C	3D	Mean	
Mean weight-gain (g), 29-56d of age						
Mash	1366	1358	1257	1152	1283	SE ±
Pellets	1420	1399	1320	1251	1348	16.48*
Mean	1393	1378	1288	1201		
		SE ± 13.15***				
	SE of mean in the body of table ± 21.2					
Mean food intake (g), 29-56d of age						
Mash	3494	3550	3448	3364	3464	SE ±
Pellets	3664	3703	3607	3580	3639	20.2***
Mean	3579	3626	3527	3427		
		SE ± 27.8***				
	SE of mean in the body of table ± 39.2					
Food conversion efficiency (Weight-gain, g/100 g food)						
Mash	38.76	37.88	36.12	33.89	36.66	SE ±
Pellets	39.09	38.06	36.91	35.32	37.34	0.5 NS
Mean	38.92	37.97	36.52	34.60		
		SE ± 0.25***				
	SE of mean in the body of table ± 0.5					

NS = not significant; * = $P < 0.05$; *** = $P < 0.001$

effects, the MKC hemicellulase gave numerically better growth rates than Betaganase M. Chicks fed on diets in pelleted form (Table 4.12) gained significantly more weight than those fed on the corresponding diets in mash form ($P < 0.05$). There were no significant interactions.

Food consumption

Chicks fed on the diet containing 5% guar meal (3B) ate slightly more food than those fed the control diet (3A) but the difference was not significant. The food intakes at the 10 and 15% level of inclusion decreased with increasing level of guar meal in diet. Birds fed on the diet containing 15% guar meal (3B) consumed significantly less ($P < 0.05$) food than those fed on the diet (3B), which contained 5% guar meal. Neither enzyme had a marked effect on food consumption when added to the control diet (3A) but their inclusion in diets containing guar meal (3B to 3D) increased the food intake of chicks significantly ($P < 0.001$) compared with those fed on the corresponding unsupplemented diets. MKC hemicellulase had a more beneficial effect in improving the food intake than did Betaganase M at the levels used in this experiment. Chicks fed on diets in pellet form (Table 4.12) consistently consumed significantly more food ($P < 0.01$) than those fed on the same diets in mash form.

Food conversion efficiency

Chicks fed on diets containing guar meal (3B to 3D) had significantly lower ($P < 0.01$) FCE values than those fed on the control diet (3A). FCE decreased with increasing level of guar in diet. Neither enzyme significantly affected the FCE when added to the control diet (3A), but when added to diets containing guar meal (3B to 3D) each improved ($P < 0.01$) the FCE of chicks compared with those fed on the corresponding diets with no added enzymes. There were

no significant differences between the FCE values of chicks fed on diets in mash or pellet forms. There was a significant interaction ($P < 0.05$) between the effects of guar meal and enzymes.

Body composition

The moisture, protein, fat and mineral contents of chicks fed on the diets containing guar meal are summarised in table 4.13. The results were analysed statistically for possible differences in the parameters measured and the appropriate tables are set out in Appendix C3.

Moisture

Neither the inclusion of guar meal up to a dietary level of 15% nor the addition of either of the two enzymes had a significant effect on the moisture contents of chicks fed on these diets. However, differences in the moisture contents of chicks due to the dietary form were observed, chicks fed on diets in mash form had higher moisture contents ($P < 0.05$) than those fed on the same diets in pellet form.

Protein, fat and mineral contents

No significant differences in the protein, fat and mineral contents of chicks as a result of feeding diets containing up to 15% guar meal, either of the two enzymes or as mash or pellets were observed from the data analysed.

Mortality

The overall house mortality during the 8-week period was 1.92% out of which only 0.86% occurred during the experimental period (5 to 8 weeks of age). In the absence of any specific trends with the dietary treatments the mortality was considered insignificant.

Table 4.13

Body composition (per cent of live weight) of 8 week old broiler chicks fed diets containing guar meal from 29-56d of age

	Diet					
	3A	3B	3C	3D	Mean	
Moisture						
E0	63.15	62.46	63.76	61.90	62.82	SED ± 0.43
E1	62.75	62.92	63.17	62.32	62.79	
E2	61.87	62.50	61.89	61.84	62.02	
Mean	62.59	62.63	62.94	62.02		
	SED ± 0.49					
	SED in the body of the table ± 1.21					
Protein						
E0	19.75	19.35	19.57	19.72	19.60	SED ± 0.19
E1	19.28	19.88	19.14	19.28	19.40	
E2	19.73	19.41	19.32	19.65	19.53	
Mean	19.59	19.55	19.34	19.55		
	SED ± 0.22					
	SED in the body of the table ± 0.54					
Fat						
E0	13.25	14.25	12.42	14.00	13.49	SED ± 0.49
E1	14.12	13.14	13.64	14.09	13.75	
E2	14.50	14.10	14.82	14.32	14.44	
Mean	13.96	13.83	13.65	14.14		
	SED ± 0.57					
	SED in the body of the table ± 1.39					
Minerals						
E0	2.77	2.65	2.78	2.90	2.78	SED ± 0.05
E1	2.64	2.70	2.78	2.75	2.72	
E2	2.70	2.70	2.53	2.75	2.67	
Mean	2.70	2.68	2.70	2.80		
	SED ± 0.06					
	SED in the body of the table ± 0.15					

4.4 Broiler experiment 4

Object

This experiment was carried out to study the effect of the addition of Rhozyme[1] HP-150 and cholesterol to diets containing guar meal on the performance of broiler chicks.

Experimental design

The experiment was of a replicated partial factorial design where the factors were cholesterol and Rhozyme HP-150. Each additive was used singly at four dietary inclusion levels with a common control diet and at one level in combination. Each of the eight experimental diets was fed to triplicate lots of six chicks from 8 to 28d of age.

Birds and management

About 180, one-day-old female broiler chicks from the same hatch were wing-banded, housed in thermostatically controlled battery brooders and reared to one week of age on a broiler mash (Appendix B4). On the 8th day all the chicks were individually weighed and 144, selected from the middle band, were distributed to one of 24 cages according to a randomised plan so that each cage contained six chicks. The cages were fitted with food and water troughs, had raised screen floors and were located in one room in a controlled environment. Food and water were available to the chicks at all times during the experimental period of twenty days. The individual body weights of chicks and the food intakes per cage were recorded at weekly intervals. During the second week of the experiment a 4d balance trial was conducted to determine the metabolisable energy (ME) contents of the experimental diets. At the end of the experiment blood samples

1. An enzyme preparation with hemicellulase activity marketed by Rohm and Haas Company, Philadelphia.

from the two chicks per replicate (six chicks per dietary group) were collected and the plasma cholesterol concentration determined.

Diets

There were eight experimental diets. Diet 4A, the composition of which is set out in Table 4.14, was formulated to contain 20% commercial guar meal (GM-3) and served as the control. Diets 4B, 4C, and 4D were similar to diet 4A except that they contained 5, 10, and 15 g cholesterol per kg of diet respectively. Likewise, diets 4E, 4F, and 4G were similar to diet 4A but they contained 0.5, 1.0, and 1.5 g of Rhozyme HP-150 per kg of diet respectively. Diet 4H was similar to diet 4G except that, in addition to the Rhozyme HP-150, it also contained 15 g of cholesterol per kg. The cholesterol and Rhozyme HP-150 in these diets were substituted at the expense of the cellulose powder in the control diet. Each of the eight diets was fed ad libitum in mash form to triplicate lots of six chicks from 8 to 28d of age.

Results

The effects of the addition of cholesterol and of Rhozyme HP-150 to diets containing guar meal on the performance of chicks are summarized in Table 4.15. The data were examined for treatment effects by analysis of variance.

Weight-gain

Birds fed on the control diet (4A) and those fed on diets supplemented with graded amounts of cholesterol (4B to 4D) gained almost the same body weight during the 20d experimental period. Chicks fed on diet supplemented with 0.5% cholesterol (4B) were heavier, though not significantly so, than those fed on either the control diet (4A) or diets containing 1.0 or 1.5% cholesterol (4C, 4D).

Table 4.14

Composition of the basal diet

Ingredient	g/kg
Maize	276
Wheat	240
Soya bean meal	80
Guar meal (GM-3)	200
Meat- and bone-meal	50
Herring meal	50
Maize oil	50
Cellulose powder	30
$CaCO_3$	7.5
$CaHPO_4$	5
DL-Methionine	4
Mineral premix*	2.5
Vitamin premix*	2.5
NaCl	2
Choline chloride**	0.5
Determined analysis	
Crude protein %	23.5

* Compositions of mineral and vitamin premixes are given in Appendix B1.

** Contained 50% choline chloride with silica as diluent.

Table 4.15

Growth, food intake, FCE and plasma cholesterol content of chicks fed on diets containing 20% guar meal with and without supplemental cholesterol and Rhozyme HP-150

Diet	Supplement to basal diet	Liveweight (g/chick) 8d of age	Liveweight (g/chick) 28d of age	Weight gain (20-days) (g)	Food Intake (g)	FCE (g, gain/100g food	Plasma cholesterol (mg/100 ml)	ME of diet (kcal/kg)
4A	None	106±3	504±13	398^{a}±12	914^{a}	43.5^{a}±1.0	90^{a}±10	2393± 63
4B	0.5% cholesterol	105±2	516±12	411^{a}±11	908^{a}	45.2^{a}±0.8	92^{a}± 7	2502± 43
4C	1.0% cholesterol	108±2	501±16	393^{a}±17	888^{a}	44.2^{a}±1.0	141^{b}±12	2600± 35
4D	1.5% cholesterol	106±2	497±10	391^{a}± 9	917^{a}	42.6^{a}±1.8	130^{b}±15	2455±110
4E	0.05% Rhozyme HP-150	109±2	579±27	470^{b}±26	912^{a}	51.5^{b}±1.2	102^{a}± 6	2813± 2
4F	0.1% Rhozyme HP-150	105±2	605± 2	500^{b}± 4	943^{a}	53.0^{b}±1.3	106^{a}± 2	2788± 31
4G	0.15% Rhozyme HP-150	107±2	602±19	495^{b}±19	944^{a}	52.4^{b}±0.9	102^{a}± 5	2815± 29
4H	0.15% Rhozyme HP-150 + 1.5% cholesterol	103±2	600± 8	497^{b}± 6	974^{a}	51.0^{b}±0.6	317^{c}±10	2733±104

Means (±SE) bearing same superscripts are not significantly different ($P < 0.05$) in the appropriate columns

The addition of Rhozyme HP-150 to diets containing 20% guar meal increased the weight-gain of the chicks significantly ($P < 0.001$). Although the addition of Rhozyme HP-150 at all dietary levels tested (0.05, 0.1, and 0.15%) was effective in promoting growth the diet containing 0.1% enzyme (4F) gave the numerically best growth; the value was not significantly different from those of birds fed the other levels of enzyme. Cholesterol at 1.5% and Rhozyme HP-150 at 0.15% levels were not synergistic when added to diets containing 20% guar meal (4G).

Food intake

The data in Table 4.15 show that food intakes of chicks fed on the control diet (4A) and on diets containing graded amounts of cholesterol (4B, 4C and 4D) did not differ significantly although chicks fed on the diet containing 1.0% cholesterol (4C) consumed numerically less food.

The addition of Rhozyme HP-150 at the three dietary levels tested (4E to 4G) did not affect significantly the food intakes of chicks; however, a trend of increasing food intake with increasing enzyme level was noted. When the diet containing 0.15% Rhozyme HP-150 was supplemented with 1.5% cholesterol (4H) the food intakes of chicks appeared to increase although not significantly. There were no significant interactions.

Food conversion efficiency

The data on FCE of chicks fed on the control diet or on those with added cholesterol did not show any significant differences. Chicks fed on diets containing 0.5 and 1.0% cholesterol (4B and 4C) had FCE values of 45.2 and 44.2gof gain/100g food eaten respectively, values which were better than those recorded by chicks fed on either the control diet (4A) or the diet containing 1.5% added cholesterol (4D).

Birds fed on diets supplemented with Rhozyme HP-150, irrespective of inclusion rate, showed significantly better FCE than those fed on the control diet ($P < 0.001$). There were no significant differences in the FCE of chicks due to the levels of enzyme inclusion; however, chicks fed on the diet containing 0.1% Rhozyme HP-150 (4F) recorded best FCE value (53.0g gain/100 food eaten) which was about 10 units higher than the value recorded by chicks fed on the diet without supplemental enzyme (4A). There were no significant interactions.

Mortality

There was no mortality during the experiment.

Effect on plasma cholesterol

Chicks fed on the control diet (4A) had lower plasma cholesterol levels (90 $\pm$ 10mg/100ml) compared with the values (116 $\pm$ 4mg/100ml) reported elsewhere (table 4.17) for the same age and breed of chicks. Supplementation of the diet with 0.5% cholesterol (4B) did not result in any significant change in the plasma cholesterol levels of the birds. Raising the dietary cholesterol level to 1.0 or 1.5%, however, did result in increases in plasma cholesterol levels ($P < 0.01$).

The addition of Rhozyme HP-150 to diets containing 20% guar meal (4E to 4G) resulted in an increase in the plasma cholesterol levels of chicks compared with those fed on the unsupplemented diet (4A). Increasing the dietary level of Rhozyme HP-150 from 0.05 to 0.15%, however, did not result in significant differences in the plasma cholesterol levels of the chicks.

The addition of 1.5% cholesterol to a diet containing 0.15% Rhozyme HP-150 (4H) resulted in a marked increase in plasma cholesterol concentration ($P < 0.001$).

Metabolisable energy

Diet 4A had a lower ME value than those supplemented with cholesterol or Rhozyme HP-150 (4B to 4H); the latter had marked effect in improving the ME value of a guar meal-containing diet.

4.5 Broiler experiment 5

Object

This experiment was conducted to study the effect of the dietary addition of commercially available preparations of saponin, cholesterol and guar gum, both alone or in combination, on the performance of broiler chicks.

Experimental design

The experiment was of a replicated factorial design where the factors were saponin, gum and cholesterol. Each was added to the control diet, both singly and in all possible combinations. Each of the eight experimental diets was fed to triplicate lots of six chicks from 8 to 28d of age.

Birds and management

One hundred and fortyfour 8-day-old female broiler chicks were used. Their management, before and during the 20-day experimental period, selection and randomization to the 24 cages were the same as described in a previous experiment (4.4). At the end of the experiment blood samples from two chicks per cage (six chicks per dietary group) were collected and plasma cholesterol concentration determined.

Diets

There were eight experimental diets. Diet 5A, the composition of which is given in table 4.16, was formulated to satisfy the chicks' requirements for all known nutrients and served as the control. Diets 5B, 5C and 5D contained 5g saponin (BDH), 15g guar gum (SIGMA CHEM.), and 10g cholesterol per kg respectively. Diets 5E and 5F were similar to diets 5B and 5C but contained, in addition 10g cholesterol per kg. Diet 5G was also similar to diet 5B except that 15g guar gum were added per kg of diet. Diet 5H contained all

Table 4.16

Composition of the basal diet

Ingredient	g/kg
Maize	303
Wheat	270
Soya bean meal	250
Maize oil	25
Meat- and- bone meal	50
Herring meal	50
$CaCO_3$	7.5
$CaHPO_4$	5
Choline chloride*	0.5
NaCl	2
DL-methionine	2
Vitamin premix**	2.5
Mineral premix**	2.5
Cellulose powder	30
Determined analysis	
Crude protein %	23.4

* Contained 50% choline chloride with silica as diluent

** Compositions of mineral and vitamin premixes are given in Appendix B1.

the three supplements (saponin, guar gum and cholesterol) in the amounts stated above. The three supplements were substituted in the control diet at the expense of cellulose powder. Each of the eight diets was fed ad libitum, in mash form, to triplicate lots of six chicks from 8 to 28d of age. During the second week of the experiment a 4d balance trial was carried out to determine the ME values of the experimental diets.

Results

The live weights, food intakes, food conversion efficiencies and plasma cholesterol concentrations of chicks fed on diets supplemented with and without saponin, guar gum, and/or cholesterol are summarized in table 4.17. The results were examined for treatment differences by analysis of variance.

Live weight gain

The analysis for growth showed that chicks fed on the control diet (5A) gained more weight than those fed on diets containing any of the three supplements either singly or in combination. The addition of 0.5% saponin to the diet (5B) depressed the growth of chicks significantly compared with those fed on the control diet ($P < 0.001$). When 1.5% guar gum was added to diet containing saponin (5B) the growth depression increased significantly ($P < 0.001$). The addition of the other two supplements to the diet (5B) further depressed growth. Cholesterol at 1% (5D) had no beneficial effect on the growth of birds when added alone, but its inclusion in the diet containing 0.5% saponin (5E) relieved the chicks completely from the growth depression brought about by the presence of saponin in diet (5B); hence there was a significant interaction ($P < 0.001$) between saponin and cholesterol.

Table 4.17

Effect of saponin, guar gum and cholesterol supplementation in diet on growth, food intake, FCE and plasma cholesterol of chicks.

Diet	Supplement to basal diet	Live weight (g/chick) 8d of age	Live weight (g/chick) 28d of age	Weight gain (20 days) (g)	Food Intake (g/chick)	FCE (g,gain/100g food)	Plasma cholesterol (mg/100 ml)	ME_n of diet (kcal/kg)
5A	None	105^{a} ±1	615± 3	510^{a} ± 3	953^{a}	53.5^{ab} ±0.2	116^{c} ± 4	2773±12
5B	0.5% saponin	106^{a} ±2	518± 5	412^{b} ± 2	733^{d}	56.3^{a} ±1.0	112^{c} ± 6	2808±10
5C	1.5% guar gum	104^{a} ±1	459±13	355^{cd} ±13	873^{bc}	40.7^{e} ±0.9	106^{cd} ±11	2428±84
5D	1% cholesterol	105^{a} ±1	611± 3	506^{a} ± 3	936^{ab}	54.1^{ab} ±1.0	194^{a} ±17	2792±64
5E	0.5% saponin + 1% cholesterol	110^{a} ±1	618± 4	508^{a} ± 4	968^{a}	52.5^{b} ±0.7	148^{b} ± 6	2780±16
5F	1.5% guar gum + 1% cholesterol	105^{a} ±2	504±14	399^{b} ±16	850^{c}	46.9^{cd} ±1.1	121^{bc} ±12	2483±36
5G	0.5% saponin + 1.5% guar gum	105^{a} ±1	437± 9	332^{d} ± 9	694^{d}	48.0^{c} ±2.3	80^{d} ± 2	2432±105
5H	0.5% saponin + 1.5% guar gum + 1% cholesterol	109^{a} ±1	473±10	364^{c} ±11	821^{c}	44.3^{d} ±1.0	117^{c} ± 6	2538±65

Means (±SE) bearing same superscripts in the appropriate columns are not significantly different ($P < 0.05$)

The addition of 1% cholesterol to the diet containing 1.5% guar gum (5F), although increasing the growth of chicks significantly ($P < 0.1$) did not relieve them completely from the adverse effect caused by the presence of guar gum in diet. Supplementation of 1% cholesterol to the diet containing both saponin and guar gum slightly reduced the growth depression ($P < 0.05$).

Food consumption

Chicks fed on the diet containing 0.5% saponin with 1% cholesterol (5E) had the highest food intake, followed by those fed on the control diet (5A) and the diet containing 1% cholesterol (5D); these latter did not differ significantly from each other. Birds fed on the diet containing 0.5% saponin (5B) consumed significantly less food than those fed on the control diet ($P < 0.001$); food intake was further reduced by the inclusion of 1.5% gum (5G) although the difference was not significant. Chicks fed on the diet containing 1.5% guar gum (5C) consumed significantly less food than those fed on the control diet ($P < 0.05$). The addition of 1% cholesterol to the diet containing gum (5F) resulted in a reduction in food intake. This was further reduced by the inclusion of 0.5% saponin in the diet (5H), although the differences were not significant. There was a significant interaction between saponin and cholesterol ($P < 0.001$).

Food conversion efficiency

From the data (Table 5.2) it is seen that the FCE of chicks fed on diets containing 0.5% saponin (5B), 1% cholesterol (5D) or both together (5E) did not differ significantly from those fed on the control diet. Inclusion of 1.5% guar gum in diet (5C), however, markedly reduced the efficiency of the utilisation of the food ($P < 0.001$). This depression in FCE was overcome to an appreciable extent by the inclusion of 1% cholesterol (5F). The inclusion of 0.5% saponin in

the diet containing guar gum (5G) also markedly improved the FCE because the food intake was reduced.

Effect on the dietary ME

Diets containing guar gum either alone (5C) or in combination with cholesterol (5F), saponin (5G) or both (5H) had lower ME values than either the control diet (5A) or the diets containing saponin and/or cholesterol (5B, 5D, 5E).

Effect on plasma cholesterol

Feeding the diet supplemented with 1% cholesterol (5D) to chicks resulted in higher plasma cholesterol concentrations in comparison to birds fed on the control diet ($P < 0.001$). Diets containing 0.5% saponin (5B) or 1.5% guar gum (5C) tended to lower the plasma cholesterol levels, guar gum being more hypocholesterolemic than saponin at the levels used. Saponin and guar gum, when fed together (5G), significantly lowered the plasma cholesterol level of chicks compared with those fed on the control diet ($P < 0.01$). However, the level was restored to that of the control-fed chicks by the inclusion of 1% cholesterol (5H). Guar gum at 1.5% level (5F) appeared to be more effective than 0.5% saponin (5E) in reducing the rise in plasma cholesterol brought about by feeding of 1% cholesterol (5D).

4.6 Discussion

In the first broiler experiment chicks fed on the diet containing 10% GM-1 had poorer growth rate and FCE than those fed either on the control diet or on the diet containing 5% GM-1. Reference to Appendix B5, however, does not indicate any apparent nutritional deficiency in the guar meal-containing diet. The poor performance of chicks given diets containing guar meal has been observed by other workers (Sathe and Bose, 1962; Ogra et al., 1963; Vogt and Penner, 1963; Vohra and Kratzer, 1964a; Keppens, 1964). The main difference in the composition between the control and the guar meal diets was the contents of guar meal substituted mainly for soya bean meal on a equal nitrogen basis. To minimise any chance of improper mixing special care was taken in mixing of the dietary ingredients and particularly the micronutrients which were premixed with a portion before adding to the main diet.

Although this sample of guar meal (GM-1) was found positive in a trypsin inhibitor test, toasting the meal at 107°C for 2 hours did not measurably improve its nutritional value for the chick. This suggests that the presence of a trypsin inhibitor, which was reported to be heat labile (Hooper and Couch, 1971), is not a major factor in determining the nutritional value of guar meal for the chick. Guar meal was not improved on autoclaving for rats (Borchers and Ackerson, 1950) and for chicks (Nagpal et al., 1971). The absence of a beneficial response on growth of chicks due to increasing the dietary methionine to twice the recommended level (NRC, 1971) in a diet containing 10% GM-1 (4.1) or 15% GM-2 (4.2) excludes the possibility that a deficiency of methionine was responsible for the poor performance of chicks under those circumstances.

Another sample of guar meal (GM-2) was also not improved by heat treatment. The possible involvement of vitamin(s) in the utilisation of the meal was also ruled out as no beneficial effect on the performance of chicks of the addition of higher vitamin doses, three times the recommended levels (NRC, 1971), was observed.

In view of the results obtained in the two broiler experiments (4.1 and 4.2) and from the observation that droppings from chicks fed on guar meal-containing diets were generally sticky, it was felt that the poor performance of chicks may be due to the presence of the residual gum in the meal. Support for this view was available from the findings of the third broiler experiment where supplementation of either of the two enzyme preparations, MKC hemicellulase and Betaganase M in diets containing guar meal was found to have definite beneficial effects on the performance of chicks, although the growth depression was not completely alleviated. The improvement in the nutritional value only in the case of diets containing guar meal could well be due to the ability of these enzymes to hydrolyse the gum present in the meal. The beneficial effect of the two enzymes, however, remained limited and almost constant at all levels of dietary guar meal thereby indicating either their limited capacity to hydrolyse the gum or the possible presence of some other toxic factor in the meal.

Further experiments to improve the nutritive value of guar meal for chicks and to identify the toxic factor(s) were carried out and the results are summarised in Tables 4.15 and 4.17.

Feeding of a diet containing 0.5% saponin or 1.5% guar gum or both together to chicks from 8-28d of age was found to have definite adverse effects on their growth, food intake and plasma cholesterol contents. Simultaneous feeding of 1% cholesterol in the

diet brought back the plasma cholesterol level but not the body weight of chicks to the same as those fed on the control diet. These observations corroborate in part the findings of Fahrenbach et al. (1966) who observed a hypocholesterolemic effect of adding 3% guar gum in the diet of chicks although the growth was reported to be affected only slightly. Feeding of a diet containing 20% GM-3 (Table 4.15) appeared more hypocholesterolemic than those containing either 0.5% saponin or 1.5% guar gum (Table 4.17) suggesting either a gum content in the guar meal diet of more than 1.5% or the possibility for the presence of a toxic saponin in the meal.

The observation that addition of Rhozyme HP-150 to the diet containing 20% GM-3 improved its utilisation by the chick (Table 4.15) holds special significance. In view of the fact that this enzyme is known to possess a specific activity against gums and mucilages and the beneficial effect brought about by its inclusion in diets containing guar meal it seems almost certain that the presence of gum in the meal is a major factor responsible for the adverse performance of chicks when fed on diets containing guar meal. The low ME values obtained for diets containing guar gum or the meal (Tables 4.15 and 4.17) also support this hypothesis and offer an explanation for the poor performance of chicks in earlier broiler experiments (4.1, 4.2 and 4.3), where the dietary ME may have been a limiting factor.

Conclusions

Inclusion of guar meal in the dietary formulations for the broiler chick exerts an adverse effect on their performance, the effect increases with the increasing dietary level of guar meal. Toasting or autoclaving of the meal, supplementation with methionine or vitamins above the acknowledged requirement levels appeared to have no measurable effect on the utilisation of the meal. Supplementation of guar meal containing diets with enzymes, Rhozyme HP-150, MKC hemicellulase and Betaganase M improved the nutritive value of the meal for the chicks, their efficacy being in the same order. Although the possibility of the presence of certain other toxic factors such as saponins may not be ruled out the presence of gum in the meal emerged as the major factor responsible for the adverse effect on the performance of chicks and for the low nutritive value of the meal.

5. EGG PRODUCTION

5.1 Introduction

Abeger (1958) reported that inclusion of guar meal in laying hens' diets had adverse effects on egg production, egg quality and FCE. An adverse effect on laying performance of birds was similarly reported by Fernandez and Santiago (1961). Bakshi et al. (1964) found decreases in egg production and egg size when a diet containing 10% raw guar meal was fed to laying hens; these effects appeared to be overcome after the meal had been heat processed. Saxena and Pradhan (1974) fed guar meal to hens of four breeds, White Leghorn, New Hampshire, Australorp and White Cornish, for a 10-week period. Performance decreased when 10% guar meal was included in a diet containing 15% protein but not in a diet containing 20% or more. These reports as well as the results of experiments described in sections 4 and 7 indicate the presence in the meal of some toxic substance (s).

Anderson (1957) noted a drop in egg production by birds fed diets containing 0.3% alfalfa saponin. Newman et al. (1958) reported reductions in the blood cholesterol of chickens fed a saponin. In an earlier experiment (4.4) feeding of 20% dietary guar meal was found to depress growth, lower FCE and plasma cholesterol level of broiler chicks. Simultaneous feeding of 1% cholesterol in diet counteracted the lowering of plasma cholesterol and to some extent the growth depression caused by the feeding of 20% guar meal.

Experiments with laying hens were conducted to measure their response when guar meal is included in their diet for both short and long periods. The effects on laying performance of adding cholesterol or methionine to guar meal-containing diet were also investigated.

5.2 Laying hen Experiment 1

Object

The object of the experiment was to study the effect on egg laying performance of birds of the inclusion of a commercial sample of guar meal (GM-1) in the diet.

Birds and management

Forty twenty-week old Shaver medium-bodied white egg layers were used in the experiment. The birds were housed in individual layers cages located in a controlled environment house where the air temperature was maintained around 21°C and 15 hours of light was provided. Ten birds were assigned to receive one of four experimental diets according to a randomised plan. Egg production and food consumption for each bird were recorded.

Diets

There were four diets, 6A to 6D, the compositions and analyses of which are set out in Table 5.1. Diet 6A was formulated using practical type ingredients and keeping in view the requirements of laying hens for essential nutrients (NRC, 1971). Guar meal (GM-1) was substituted for a mixture of soya bean meal and sawdust (85:15) at 10, 20 or 30% dietary levels to obtain diets 6B, 6C and 6D respectively. The diets were offered to the birds *ad lib.* as mashes for a 4-week period. Water was available at all times.

Egg-yolk colour

After the birds had been on the experimental diets for two weeks, ten eggs from each dietary group were taken and stored at 4°C for fourteen days. The eggs were then examined for yolk colour using a Roche Yolk-colour fan.

Table 5.1

Composition and analyses of diets (g/kg)

Ingredient	Diet 6A	6B	6C	6D
Maize meal	270	270	270	270
Wheat meal	300	300	300	300
Soya bean meal mix*	300	200	100	-
Guar meal (GM-1)	-	100	200	300
Maize oil	30	30	30	30
$CaCO_3$	60	60	60	60
$CaHPO_4$	25	25	25	25
NaCl	7	7	7	7
Vitamin premix**	2.5	2.5	2.5	2.5
Mineral premix**	2.5	2.5	2.5	2.5
DL-Methionine	2	2	2	2
Choline chloride***	1	1	1	1
Analyses				
Dry matter	892	893	890	891
Crude protein	168	172	171	173
Calcium	32	34	32	33
Phosphorus	7	7	6.9	7

* Contained soya bean meal and sawdust, 85:15 parts respectively

** The composition of vitamin and mineral premixes are set out in Appendix B1

*** Contained 50% choline chloride in silica as base.

Results

Data on egg production and food intake of birds, and egg yolk-colour index are summarised in Table 5.2. The egg production performance as affected by the dietary inclusion of guar meal is shown in Figure 5.1.

It can be seen that dietary inclusion of guar meal resulted in decreased egg production from birds, the depression increasing with increasing dietary level of guar meal. The decline in the egg production of hens was noticed almost immediately they were fed on the diets containing the meal and increased with time. The food intake of birds fed diets containing guar meal, although numerically less than that of birds fed on the control diet, was not seriously affected. The yolk-colour index also fell with the inclusion of guar meal in the diet.

Table 5.2

Effect of inclusion of guar meal in diet on the laying performance of birds

Weekly egg production (%)	Diet 6A	6B	6C	6D
1	57.1	52.8	44.3	41.4
2	54.3	52.8	31.4	2.8
3	50.0	32.8	30.0	1.4
4	54.3	30.0	32.8	5.7
Mean egg production (4-week period) %	53.9 ± 1.5	42.1 ± 6.2	34.6 ± 3.3	12.8 ± 9.5
Food consumption (g/bird/day)	117	110	106	105
Yolk-colour index	5.0 ± 0.15	3.8 ± 0.20	3.4 ± 0.22	3.7 ± 0.21

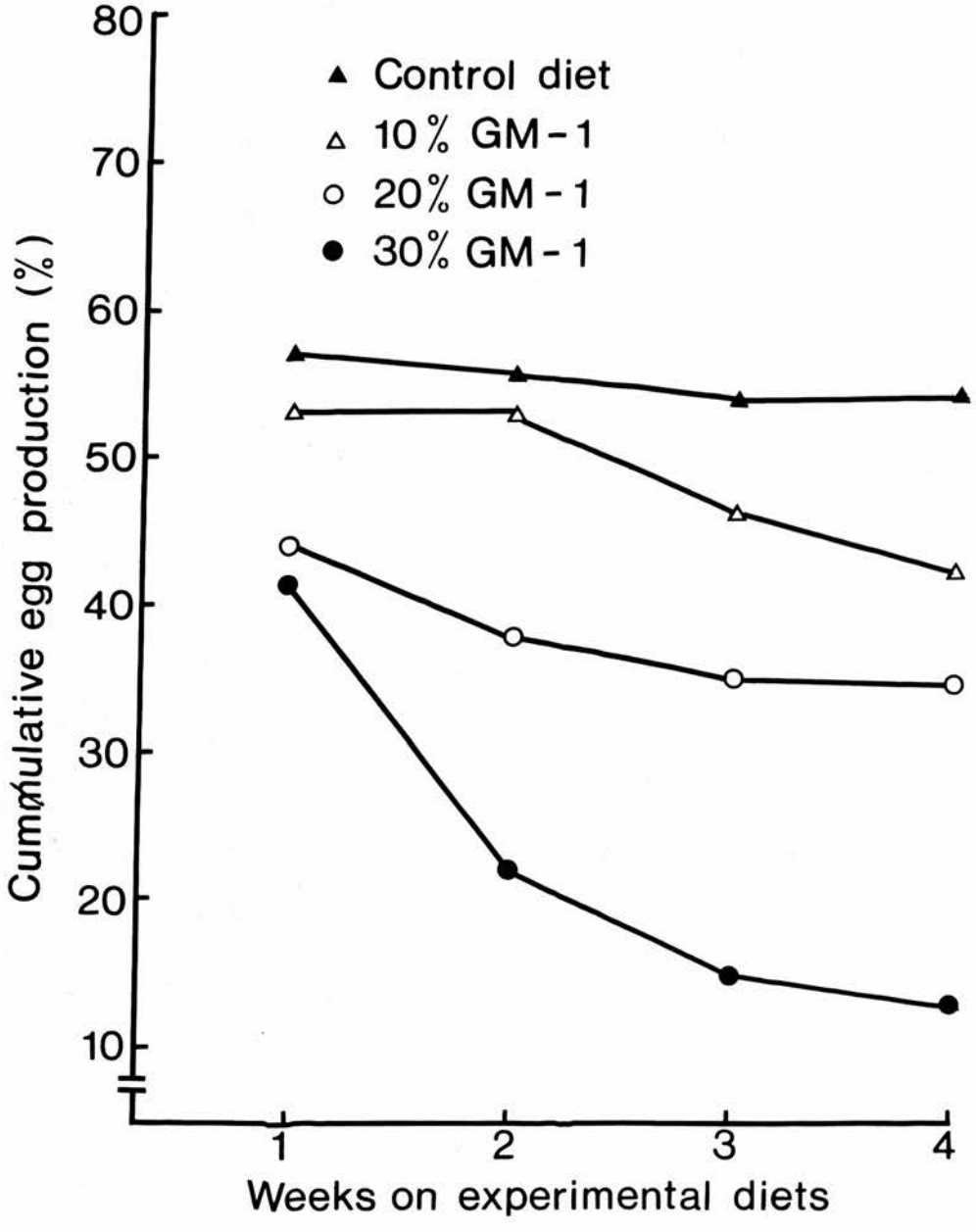


Fig 5.1 Effect of inclusion of guar meal in diet on egg production.

5.3 Laying hen Experiment 2

Object

Effect of cholesterol supplementation in diet containing guar meal on the laying performance of hens.

Birds and management

Thirty eight 28-week old Shaver medium-bodied white egg layers were housed in individual layers cages located in a controlled environment house where the room temperature was maintained around 21°C and 15 hours of light was provided. The birds were assigned to four groups according to a randomised plan. Two of the groups had nine birds while the other two had ten birds in each. Four diets were assigned randomly within four groups and offered to birds *ad lib.* as mash for a 4-week period during which records of food consumption and egg production were kept.

Diets

Diet 6C (Table 5.1) containing 20% GM-1 served as a control diet in this experiment. Three more diets were prepared by supplementing 0.5, 1.0 and 2.0% cholesterol in the control diet (6C).

Egg-yolk colour

During the final two weeks of the experimental period fifteen eggs from each of the four dietary groups were taken and stored at 4°C for a 2-week period. The eggs were then examined for yolk colour using a Roche yolk-colour fan.

Results

Data on mean egg production, food intake and yolk-colour index obtained in the experiment are summarised in Table 5.3. The per cent egg production of birds as influenced by the dietary treatments is shown in Figure 5.2.

It can be seen that the birds fed on the diet containing

Table 5.3

Effect of cholesterol supplementation in guar meal-containing diets on the laying performance of hens

	Diet			
	6C	6C+ 0.5% cholesterol	6C+ 1.0% cholesterol	6C+ 2.0% cholesterol
Weekly egg production (%)				
1	69.8	67.1	69.8	65.7
2	41.3	60.0	63.5	60.0
3	55.6	71.4	73.0	61.4
4	50.8	61.4	73.0	72.9
Mean egg production (%)	54.4 ± 4.0	65.0 ± 2.6	69.8 ± 2.2	65.0 ± 2.9
Food consumption (g/bird/day)	96	94	98	100
Yolk-colour index	3.9 ± 0.22	4.5 ± 0.19	4.7 ± 0.16	4.5 ± 0.16

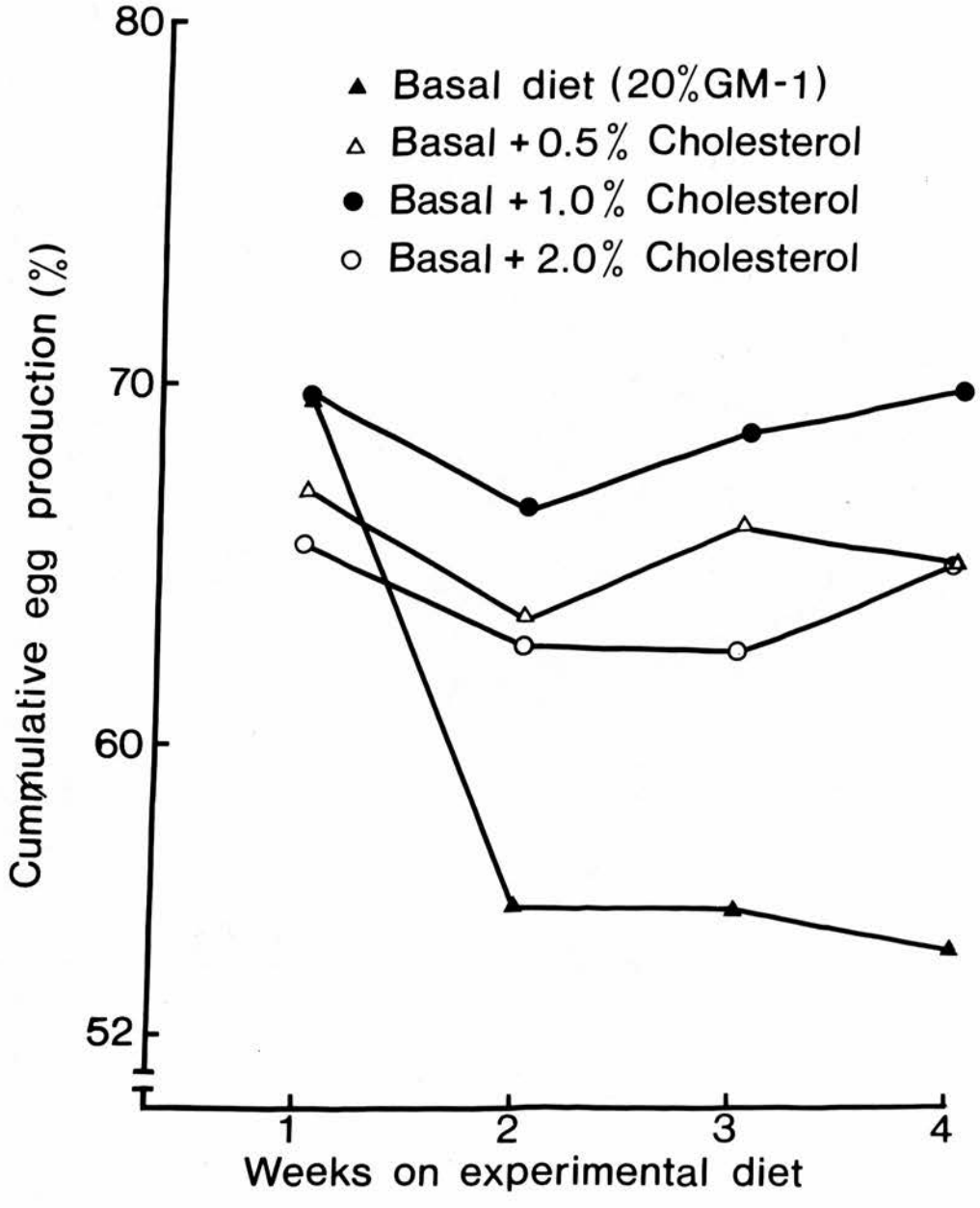


Fig 5.2 Effect of cholesterol supplementation, in guar meal containing diet, on egg production.

20% guar meal (6C) had the lowest egg production, lower than those fed on similar diets but with added cholesterol. The food intake of hens fed on different diets, however, remained the same. The yolk-colour index of eggs from hens fed on diet 6C was slightly lower than those from hens fed on diets containing cholesterol.

5.4 Laying hen Experiment 3

In an earlier experiment (5.2) inclusion of GM-1 in the diet of laying hens was found to have adverse effects on their performance. The experiment was carried out for a short period although the meal was fed at fairly high dietary levels. It was considered worth investigating the effect of the dietary inclusion of relatively lower levels of guar meal on the long term laying performance of birds.

Object

To study the laying performance of two breeds of hens offered guar meal-containing diets as mash or pellets during the laying stage. Effect of addition in diet of excess methionine on the utilisation of guar meal by the hens was also investigated.

Experimental design

The experiment was designed as a 2 x 3 x 4 factorial where the factors were breed, dietary form and the level of guar meal respectively. Treatments were allocated randomly within blocks of modules, each consisting of twelve single battery cages arranged in three tiers. The cages were 305mm wide by 460mm deep and equipped with individual food troughs and nipple drinkers.

Birds and management

700 Warren SSL and 700 Babcock B300 pullets were wing-banded at one day of age. They were housed alternately in 36 pens (3.72 m^2) and reared to 19 weeks of age. The pens had a layer of wood shavings on the floor and were situated in a house with controlled environment. Infra-red brooding lamps were provided for the first 7d period. Temperature and ventilation were maintained according to the instructions of the breeders.

The birds were individually weighed at 19 weeks of age and

then 576 birds from each of the two breeds transferred into single bird battery cage units according to a previously randomised plan. Warren and Babcock hens were housed in alternate cages side ways. Single modules at the ends of the battery cage units were also filled similarly although these birds played no part in the experiment other than to maintain a uniform environment for the experimental birds housed in the adjacent modules. Each of twelve diets (Table 5.4) was offered ad lib. to 48 birds of both breeds. Egg production was recorded daily and all eggs laid on 2 days per week were weighed individually. Food consumption for each bird was recorded on a 28-day basis. The data were collected until the birds were 73 weeks of age, when all birds were individually weighed. Mortality was also recorded.

Diets

During the rearing period all birds were fed on common starter and grower diets, the compositions and analyses of which are set out in Appendix B7. Four basal diets were used in the laying period. Diet 7A was formulated to supply the hens' requirements for known nutrients (NRC, 1971) and served as a control. Diets 7B, 7C and 7D were similar to diet 7A except that they contained 50, 100 and 150 grammes guar meal (GM-2) per kg respectively substituted for part of the soya bean and wheat meals. All the diets were calculated to be isocaloric and isonitrogenous. Each of the four basal diets were fed in three forms, mash (M), pellets of 5mm diameter (P), and mash plus 0.5% DL-methionine (M+M) added over the recommended level of 0.28% (NRC, 1971), thereby increasing the total number of diets to twelve. All diets were analysed for their proximate components and found to confirm the calculated values. The composition of the four basal diets together with the determined

Table 5.4

Composition and analyses of layers diets (g/kg)

Ingredient		Diet 7A	7B	7C	7D
Wheat meal		645	635	625	615
Soya bean meal		207	167	127	87
Guar meal (GM-2)		-	50	100	150
Maize oil		35	35	35	35
$CaCO_3$		71	71	71	71
$CaHPO_4$		30	30	30	30
NaCl		5	5	5	5
Vitamin premix*		2.5	2.5	2.5	2.5
Mineral premix*		2.5	2.5	2.5	2.5
DL-Methionine		1.0	1.0	1.0	1.0
L-Lysine		1.0	1.0	1.0	1.0
Analyses					
Crude protein	(M	159	160	160	159
	(P	158	161	160	158
	(M+M	160	162	162	161
Ether extract	(M	42	48	45	43
	(P	48	42	43	45
	(M+M	45	43	44	42
Calcium	(M	33	34	35	33
	(P	33	33	33	35
	(M+M	34	35	34	34
Phosphorus	(M	6.8	6.7	6.8	6.9
	(P	6.9	6.9	6.8	7.0
	(M+M	6.7	6.7	6.7	7.0

* Compositions of vitamin and mineral premixes are set out in Appendix B1
M= Mash; P= Pellets; M+M= Mash + DL-Methionine

crude protein, ether extract, calcium and phosphorus contents in the twelve diets are set out in Table 5.4.

Results

Data on mean egg production, egg mass, food consumption and live weights of hens fed on different diets during the experimental period of 54 weeks are set out in Table 5.5. The results were analysed for the treatment effects using standard analysis of variance methods.

Egg production

It can be seen (Table 5.5) that the number of eggs produced per hen during the experimental period decreased significantly ($P < 0.001$) when diets containing guar meal were offered as mash or pellets. When mash diets containing 5% GM-2 with excess methionine were offered the egg production was equal to or approached those of birds fed on the control diet. Supplementation of diets containing 10 or 15% guar meal with excess methionine significantly improved ($P < 0.01$) the egg production of hens of both breeds compared to those fed on the appropriate diets as mash or pellets without excess methionine. The effect of dietary inclusion of guar meal and of excess methionine on the per cent egg production of birds (combined breeds) during the experimental period is shown in Figure 5.3.

Although the birds of both breeds, Warren SSL and Babcock B300 responded in almost the same way to the inclusion of guar meal in diet (Figure 5.4), the production was most affected when Babcock hens were fed 10 and 15% guar meal in pelleted diets (Table 5.5).

Egg weight and egg mass

Egg weight was also adversely affected by the inclusion of guar meal in diet. The mean egg weight decreased significantly ($P < 0.05$) with increasing level of guar meal in diets fed both as

Table 5.5

Mean egg production, egg mass, food consumption and live weight data of hens during the experimental period.

		Warren SSL				Babcock B300				SED
	Diet/form	7A	7B	7C	7D	7A	7B	7C	7D	
Mean egg number/hen	(M	253.3	183.4	163.2	126.6	239.7	213.6	159.5	122.4	)
	(P	231.0	189.2	171.2	136.2	250.3	192.4	137.5	96.8	) 11.94
	(M+M	236.9	243.1	204.6	210.2	265.9	245.0	227.9	217.8	)
Mean egg weight (g)	(M	58.1	56.3	56.2	55.6	56.2	55.5	54.0	51.7	)
	(P	58.5	56.6	56.1	55.8	57.6	55.8	53.4	52.3	) 0.69
	(M+M	59.8	59.3	59.0	58.2	58.8	58.7	57.7	57.7	)
Total estimated egg mass (kg) (Egg number x kg mean egg weight)	(M	14.75	10.36	9.31	7.11	13.52	11.91	8.78	6.35	)
	(P	13.54	10.89	9.68	7.79	14.42	10.86	7.51	5.14	) 0.72
	(M+M	14.16	14.43	12.15	12.28	15.61	14.43	13.19	12.64	)
Food consumption (kg/hen)	(M	39.95	36.47	35.57	33.94	34.99	33.34	31.56	29.47	)
	(P	39.34	27.01	37.04	34.37	35.55	32.74	31.96	29.41	) 0.78
	(M+M	39.11	39.64	38.35	38.52	35.87	35.04	34.68	34.16	)
FCE (kg egg mass/kg food intake)	(M	36.7	27.6	25.5	20.1	38.6	35.2	27.0	21.3	)
	(P	34.1	28.4	25.4	21.6	40.4	32.2	22.7	17.1	) 1.70
	(M+M	36.1	36.1	31.3	31.5	43.6	40.9	37.7	36.7	)
Liveweight at 19 weeks (g)	(M	1692	1728	1669	1712	1276	1289	1384	1296	)
	(P	1702	1758	1739	1738	1319	1302	1310	1276	) 34
	(M+M	1686	1732	1755	1680	1294	1283	1295	1294	)
Liveweight at 73 weeks (g)	(M	2178	2042	1965	1989	1551	1475	1442	1296	)
	(P	2294	2144	2182	2095	1561	1515	1425	1385	) 53
	(M+M	2059	2091	2013	2046	1571	1493	1410	1452	)
Mortality, 19-73 weeks (%)	(M	4.2	12.5	21.4	27.0	0.0	4.2	7.1	31.2	)
	(P	8.3	16.7	27.1	40.7	2.1	25.0	25.0	44.4	)
	(M+M	4.2	8.3	8.3	4.2	4.2	4.2	8.3	6.3	)

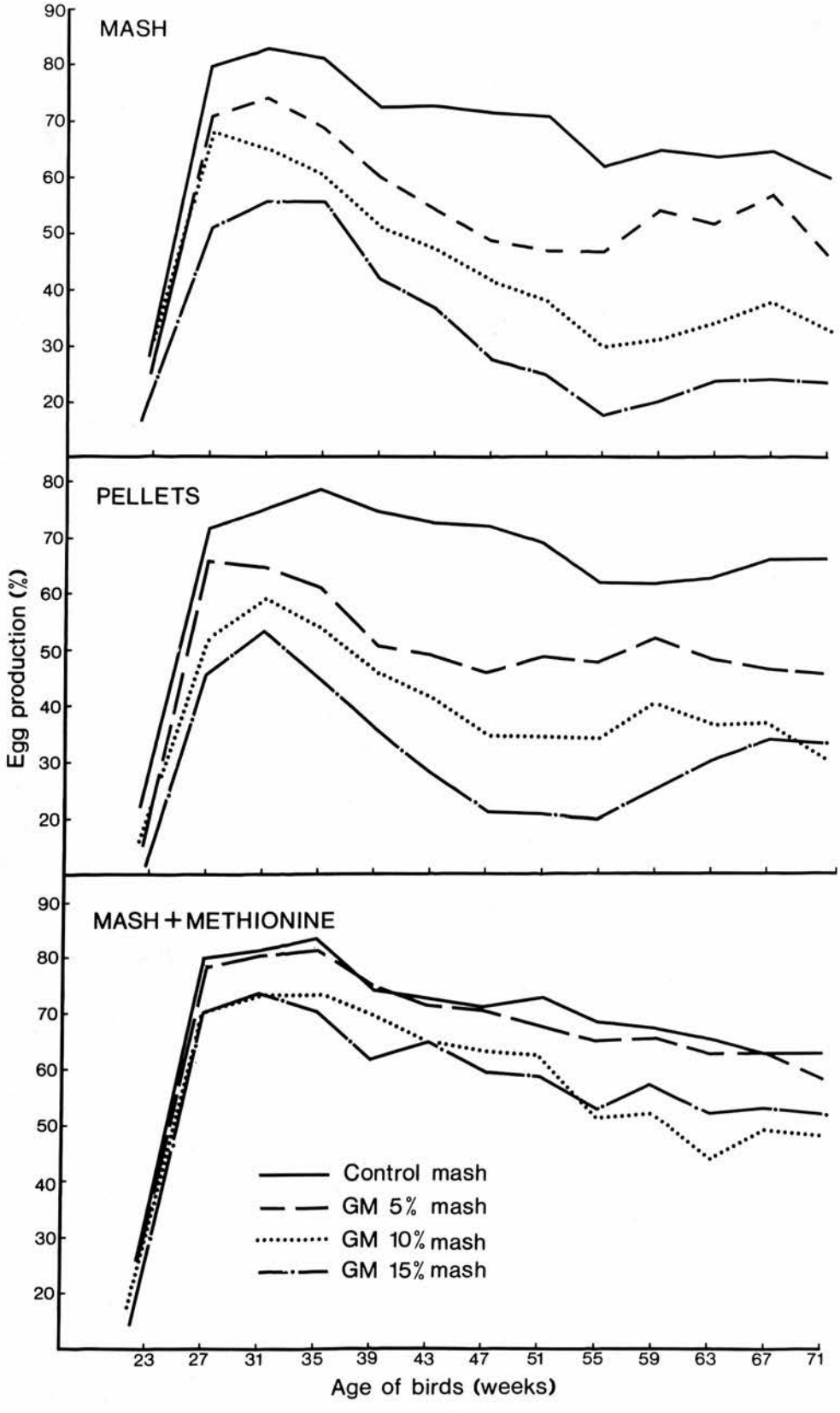


Fig 5.3 Egg laying performance of hens fed on diets containing guar meal

mash and pellets. The trend of decreasing egg weight with increasing level of guar meal was greatly reduced when the mash diets were supplemented with excess methionine. Because of the reduction in egg number and mean egg weight, the total estimated egg mass from birds fed on guar meal-containing diets offered as mash or pellets decreased significantly with increasing levels of dietary guar meal. The beneficial effect excess methionine had on egg production and egg weight, was reflected in the significantly higher ($P < 0.01$) egg mass from birds fed on such diets compared to those fed on the unsupplemented diets.

Food consumption and FCE

Birds fed guar meal-containing diets as mash or pellets consumed significantly less ($P < 0.01$) food than those fed on the control diets (Table 5.5). Food intake decreased with increasing level of dietary guar meal. Whereas the addition of excess dietary methionine significantly improved ($P < 0.05$) the food intake of birds fed on diets containing guar meal from those fed on the unsupplemented diets, it did not affect the food intake of those fed on the control diet to any measurable extent. Babcock birds consumed significantly less ($P < 0.01$) food than Warrens and so generally had significantly better ($P < 0.05$) FCE's in the corresponding dietary groups, except when fed diets containing higher levels of guar meal (7C and 7D) as pellets. FCE decreased with increasing level of guar meal in diet. Addition of excess methionine markedly improved the FCE of birds fed on guar meal-containing diets.

Live weight

Warren SSL birds were significantly heavier ($P < 0.01$) than Babcock B300 both at 19 and at 73 weeks of age and also gained more weight than the latter during the experimental period. Birds

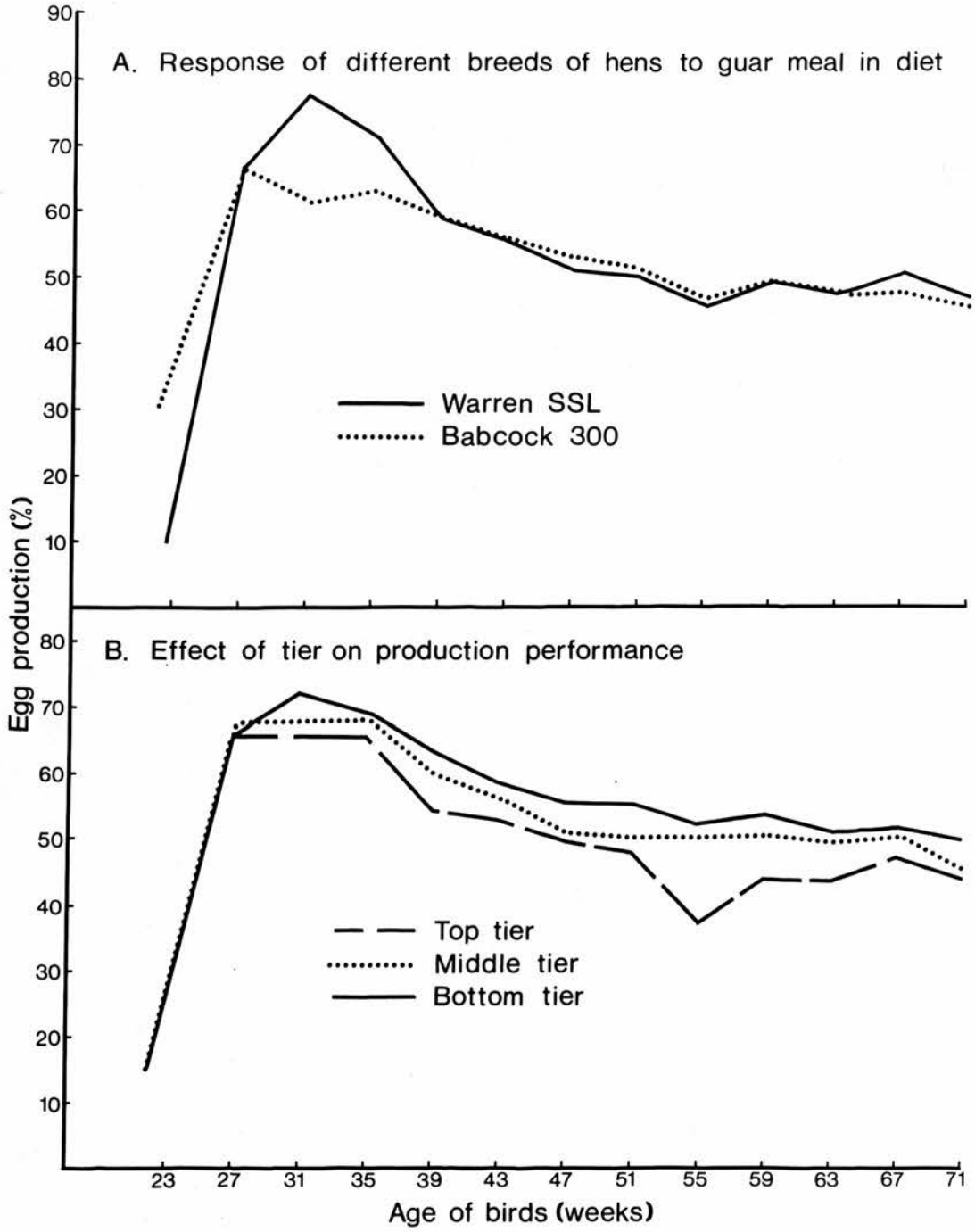


Fig 5.4 Egg laying performance of hens fed on diets containing guar meal

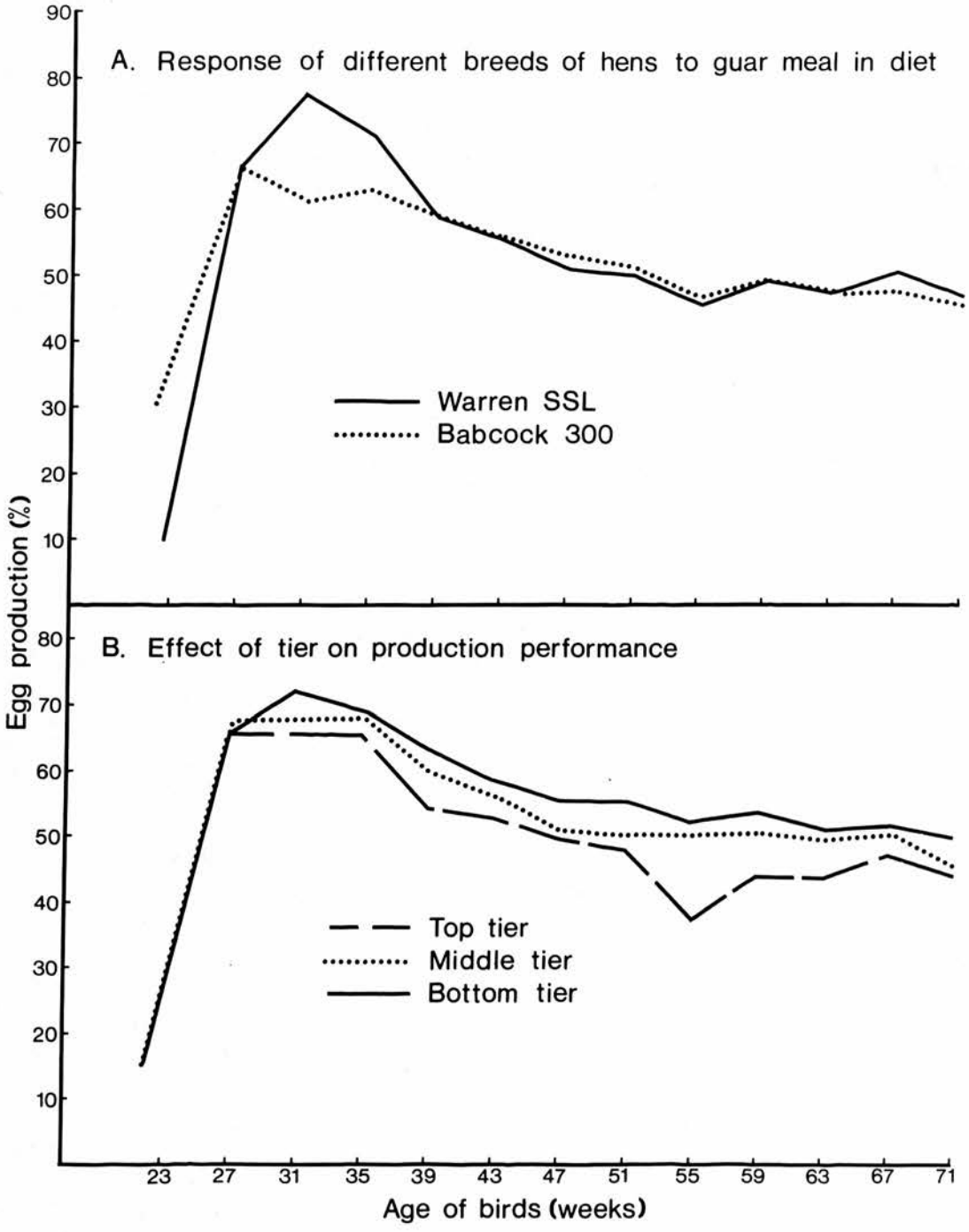


Fig 5.4 Egg laying performance of hens fed on diets containing guar meal

fed diets as pellets or with additional methionine were heavier than their counterparts fed on corresponding mash diets.

Mortality

Mortality increased with the increasing level of guar meal in diet. Birds fed diets containing 10 and 15% guar meal as mash or pellets experienced very high mortality indeed. Most of the deaths were due to emaciation through not eating the diets containing guar meal, although in a few cases nephritis, visceral gout or heart failure were diagnosed to be the cause of death. Birds fed on the guar meal diets containing excess methionine experienced very low mortality compared to those fed unsupplemented diets as mash or pellets.

5.5 Discussion

Two short term and one long term experiments were conducted to investigate the effect of dietary guar meal on the performance of laying hens. The results of the experiments demonstrated that the inclusion of as little as 5% commercial guar meal in the diets of laying hens had adverse effects on their overall performance. The observations confirm the findings of earlier workers (Abeger, 1958; Fernandez and Santiago, 1961; Bakshi et al., 1964).

The performance of birds decreased almost linearly with increasing level of guar meal in diet. Egg production started to decline within a week of the introduction of guar meal into their diets and continued to decline with time. Reference to Tables 5.1 and 5.4 does not indicate any apparent nutritional deficiency in the diet. Guar meal was substituted mainly for soya bean meal on an equal nitrogen basis, and because there were differences in the amino acid patterns of their proteins, slight differences in the amino acid composition of guar meal-containing and control diets could be expected. Whether such small differences would account for such an adverse effect on egg production after such a short period of feeding is doubtful.

It is well known that birds can regulate their food intake to some extent so as to correct minor nutritional deficiencies. In these experiments, as with those involving broilers, there appears to have been no effort made by the birds fed on the guar meal-containing diets to consume more food; instead, their food intake decreased with increasing level of guar meal in diet.

Because decreased food intake leads to decreased intake of nutrients by the bird a drop in egg production as well as a decrease in egg weight could well be expected. Besides being affected by

protein and, hence, amino acids, egg weight also decreases if the diet is deficient in metabolisable energy. The likelihood that metabolisable energy is one of the responsible factors for the adverse effects of guar meal cannot be ruled out in view of its low ME value (Section 7) for poultry.

Effect of cholesterol

In the second experiment the effect of cholesterol supplementation of diets containing 20% guar meal on the laying performance of birds was studied. Figure 5.2 shows the egg production of birds fed on the different diets. It can be seen that the egg production of birds fed on diets containing 20% GM-1 was reduced, confirming the observation of an earlier experiment (5.2). When the diet was supplemented with cholesterol the egg production, despite an initial drop, was maintained throughout the experimental period and tended to have an upward trend.

The ability of cholesterol to counteract the adverse effect of dietary guar meal on egg production could be of considerable nutritional importance. It is not to be expected that feeding cholesterol will correct a deficiency of protein or of energy. Cholesterol, however, has been reported to combine with ingested saponins in the intestinal tract (Newman et al., 1958; Griminger and Fisher, 1958) to form insoluble nutritionally-neutral complexes which are ultimately excreted. Most of the physiological effects of saponins are reported to arise from their surface activity and their ability to form complexes with sterols and proteins (Birk, 1969). It appears likely, therefore, that guar meal contains saponin(s) which are probably associated with the protein fraction of the meal. If these saponins react with the dietary protein in the birds' gut it could account, at least in part, for the poor utilisation of guar

meal. When the diet containing guar meal is supplemented with cholesterol either the guar meal saponins react with cholesterol preferentially or their ability to react with the ingested protein is reduced. In either case the net result is to make the protein more available to the bird.

This hypothesis would also offer an explanation of the findings of Saxena and Pradhan (1974) who observed that the deleterious dietary effects of guar meal were more pronounced when the diets being fed contained marginal amounts of protein.

Effect of methionine

Supplementation of guar meal-containing diets with methionine has been found to have significant beneficial effects on food intake, egg production and FCE of hens in the experiment described under 5.4. The role of methionine in the improved utilisation of guar meal by hens appears to be more than simply overcoming a dietary deficiency of the sulphur-containing amino acids. The remarkably high demand for methionine indicates its involvement in the detoxification of certain substances present in the guar meal. The high mortality of birds fed on methionine-unsupplemented (though otherwise adequate) guar meal-containing diets may be a result of the accumulation of certain toxic substances in the body. Oke (1964) reported the HCN content of the young guar beans to be from 40 to 70mg/100g dry matter. Guar meal samples used during the course of these investigations were found to contain from 5 to 17mg HCN/100 gramme.

Ingested cyanide is rapidly absorbed from the gastro-intestinal tract. Cystine has been reported to react directly with cyanide to produce 2-amino-4-thiazolinecarboxylic acid (Wood and Cooley, 1956). It may be that when guar meal is fed to hens dietary

cystine is involved in the metabolism of cyanide present in the meal, thereby increasing the dietary requirement for methionine. When the two amino acids are present in diets at the recommended (NRC, 1971) levels only, a deficiency could still be expected if food intake was low. Also, if the laying bird has a specific requirement for cystine, any detoxification reactions involving this amino acid will undoubtedly raise the level of cystine required for optimal performance. Moreover the decreased absorption of methionine in the presence of guar gum, as observed by Katoch et al. (1971) in an in vitro study, may further accentuate a deficiency of this amino acid due to the presence of the residual gum in the meal.

Conclusions

Inclusion of guar meal in the diet of laying hens had adverse effects on their performance. The adverse effects include decline in the rate of egg production, reduced food intake and a decrease in egg weight. Increasing the dietary guar meal level to more than 10% resulted in high mortality. Feeding 1% cholesterol in a diet containing 20% guar meal appeared to help maintain egg production. Increasing the dietary methionine level to about 0.8% in diets containing up to 15% guar meal significantly increased the performance of laying hens and reduced the mortality.

6. PROTEIN QUALITY ASSAY

6.1 Introduction

Because foodstuffs are used for biological purposes, such as the production of meat or eggs, it seem logical that a biological method should be employed for the evaluation of the quality of their protein. However, a considerable drawback to a biological means of assessment is the knowledge that other dietary and environmental factors may influence the value obtained. Ideally it would be desirable to define the protein chemically and calculate its value under any circumstances.

Despite a considerable amount of effort having been directed at correlating the various biological and chemical methods that have been used for protein evaluation - the review by McLaughlan and Campbell (1969) cites over 150 references - no "best" method has emerged. However amongst the various methods employed for evaluating dietary protein the determination of net protein utilisation (NPU), which measures the proportion of the ingested nitrogen retained in the body, offers a useful and reliable method for the assessment of the protein quality of a feedstuff. Derived from the Thomas-Mitchell procedure for the determination of biological value (Thomas, 1909; Mitchell, 1924) it was originally called net protein value (Bender and Miller, 1953) and measured the proportion of dietary nitrogen that was retained by rats. It was renamed NPU by Miller and Bender (1955) and is defined by the following equation.

$$NPU = \frac{Bf - (Bk - Ik)}{If}$$

Where Bf = body N at the end of test period of animals fed on the test diet.

Bk = body N at the end of test period of animals fed a diet containing no protein.

If = N intake of animals fed on the test diet, and

Ik = N intake of animals fed on the non-protein diet.

NPU can, by definition, be derived either by estimating carcass gain from faecal and urinary excretion (Rippon, 1959; Henry and Toothill, 1962) or directly from carcass analysis (Bender and Miller, 1953a). A full description of the methodology has been given by Miller (1963).

The technique involves the comparison of the nitrogen retention of a control-fed group of animals with that of the test-fed group. The animals in the control group are fed a diet composed of purified ingredients, containing no protein, while another group of animals is fed a test diet simultaneously and under identical conditions. The test diet is generally prepared by substituting the protein source under study, at the expense of glucose in the control diet, at a level such that it provides sufficient protein for maintenance and also permits a little growth. The differences in the body nitrogen of animals in the two groups and the nitrogen intake of the test animals are then used to calculate the NPU value of the protein source according to the equation given earlier.

The applicability to chicks of the carcass analysis method for the determination of NPU has been established (De Muelenaere et al., 1960; Summers and Fisher, 1961). It has been used by many workers for evaluating a number of commonly used protein-rich feedstuffs for poultry. The dietary protein level at which the feedstuff should be evaluated is a factor of considerable importance. For example NPU has been shown to decrease as the dietary protein level increases and a level of substitution which gives a dietary protein content of 13% has been recommended (Summers and Fisher, 1961).

Fisher and Griminger (1969) reaffirmed that a 13% dietary protein level reflected more accurately the growth potential of the test protein for chicks. In the present series of experiments, therefore, the protein sources were evaluated at a 13% dietary protein level.

A close relationship has been shown to exist between the body-water and -nitrogen contents of chicks of the same age reared under similar dietary and environmental conditions (De Muelenaere et al., 1960; Summers and Fisher, 1961). Once this relationship for a representative sample of chicks is established, the body-nitrogen content of the rest of the chicks reared under identical conditions can be estimated from their moisture contents only. However, in the present series of experiments the use of carcass-water and -nitrogen relationship was limited to the individual dietary groups. Out of the four replicate groups under any one dietary group, chicks from three were simply dried to determine body-water contents whereas those from the fourth replicate group, were used both for moisture and nitrogen determinations.

6.2 General procedure

Birds and management In all the NPU experiments described here male broiler chicks, Ross I or Marshalls' were used. In any one trial a sufficient number of one-day old chicks (about 40% excess of requirement) were obtained from the same hatch, wing-banded, housed in thermostatically controlled battery brooders and reared to 7d of age on a broiler mash (Appendix B 4). At the end of the pre-experimental period the chicks were fasted overnight and weighed individually to the nearest gramme. The required number of chicks from the middle band were selected and distributed, according to a previously randomised plan, to cages so that each cage contained five chicks. The cages had raised wire-screen floors, were fitted with food and water troughs and located in a controlled environment house.

Diets A basal diet composed of purified feed ingredients, vitamins and minerals was formulated and served as the non-protein control (Table 6.1). The required number of test diets were mixed by substituting the protein source(s), weight by weight, for glucose in the basal diet so as to provide diets containing about 13% crude protein. Each of the experimental diets were then offered *ad lib.* to quadruplicate lots of five chicks for a two week period. Considerable care was taken to correct for any food spillage and the net food intake per pen was calculated. All diets were analysed for protein contents (N X 6.25) and the calculated values confirmed.

Carcass moisture At the end of the feeding period all the chicks were fasted overnight to empty their digestive tracts, weighed individually, and then killed using laboratory gas to avoid loss of blood. Carcasses from three of the four replicate groups were placed on aluminium dishes, dried to constant weight in a forced-draft oven

Table 6.1

Composition of the basal diet

Ingredient		g/kg
Maize starch		280
Mineral premix*		59
Dextrin		50
Cellulose powder**		30
Maize oil		30
Vitamin premix***		2.5
Choline chloride		2
Glucose monohydrate	to	1000 g

* Composition of mineral premix is set out in Appendix B 2

** Cellulose powder was replaced by glucose monohydrate in test diets containing protein source.

*** Composition of vitamin premix is set out in Appendix B 3

at 85°C, and the loss in weight was recorded as moisture. To facilitate drying several incisions were made in the abdomen, thorax and skull. Carcasses from the fourth replicate were similarly incised, wrapped in Whatman No. 1 filter paper (24 cm diameter) and dried to constant weight. The dried carcasses from this group were used for nitrogen determinations, whereas those from the previously dried three groups were discarded.

Carcass nitrogen The dried carcasses from the fourth group, together with the stained portion of the filter paper, were transferred to separate conical flasks (ca. 2 litres) and about 300-600ml of conc. HCl, depending on the weight of carcasses, were added. The flasks were allowed to stand for 3-4 days during which time they were occasionally shaken. Any undigested portions were then dissolved by gentle heating. The contents of the flask were made up to volume (5 litres) and nitrogen determinations were carried out on triplicate aliquots by the Kjeldahl procedure. Total carcass-nitrogen was thus determined. Knowing the water to nitrogen ratio of chicks in this replicate and the water contents of chicks in the other three replicates, the nitrogen contents of these latter groups were estimated.

6.3 NPU experiments 1 and 2

Object The object of experiment 1 was to evaluate a sample of commercial guar meal (GM-1) for its protein quality in terms of NPU for growing chicks. The effect of processing the meal and of amino acid supplementation on its NPU value was studied in the second experiment.

Processing of guar meal

Extraction with water Guar meal was extracted with 12 volumes of water at 40°C (pH 6.5) for 16hr with occasional stirring and then filtered through cheese cloth. The residue was similarly extracted with a fresh amount of water and again filtered. The material was spread on to the aluminium foil-lined metal trays in layers of about 20mm thickness and dried in a forced-draft hot-air oven at 60°C. The dried cakes were ground finely before addition to the diets. The chemical composition of the processed meal is set out in Appendix B 6.

Toasting The toasted guar meal was prepared by heating the meal, in layers of about 20mm thickness, at 107°C for 2 hours in a hot-air oven.

Autoclaving Guar meal was autoclaved at 121°C for 30 min as described earlier (expt. 4.2).

Birds and management 40, one-week old Ross I male broiler chicks were used in experiment 1 and 140, of the same strain, sex and age, in experiment 2. The procedure for the selection and randomisation of chicks, into groups of five was as described earlier (6.2). They were housed in separate compartments in thermostatically controlled battery brooders. The chicks were weighed before and after the experimental period and food consumption per pen during this time was recorded.

Diets The composition of the protein-free diet (8A and 9A) used in experiments 1 and 2 is given in table 6.1. Only one test diet (8B) containing commercial guar meal, at a level providing approximately 13% protein, was used in experiment 1. In the second experiment, seven test diets (9B to 9G) were formulated. Diets 9B, 9C and 9D contained samples of guar meal which had been water-extracted, toasted or autoclaved respectively, each added at a level designed to provide approximately 13% protein in diet. Diet 9E and 9F were similar to diet 9D in that they contained autoclaved guar meal, but were supplemented with 1% lysine and 0.75% methionine respectively. Diet 9G was also similar to diet 9D but supplemented with both 1% lysine and 0.75% methionine. All the diets were fed ad lib. to quadruplicate lots of five chicks for a 2 week experimental period.

Analysis At the end of the feeding period chicks from each pen were sacrificed and processed according to the details given earlier (6.2), and carcass nitrogen contents determined.

Results Details of live-weights, food and nitrogen intakes and the body-water and -nitrogen contents of chicks fed on different diets together with the NPU values of the protein sources studied are presented in Table 6.2. The values reported are the means of the four replicated groups of five chicks.

The low NPU value (9.8 $\pm$ 1.2) of guar meal obtained in the first experiment, indicates very poor utilisation of its protein by the growing chick. When fed a diet containing approximately 35% GM-1 (8B) as the only source of protein for a 2 week period, the chicks could not maintain their body weights.

In the second experiment when water-extracted guar meal was fed to chicks as the only dietary protein-source (9B), about half of the chicks died well before the end of the 2 week feeding

Table 6.2

Live-weights, food and nitrogen intakes, body-water and -nitrogen contents of chicks and the NPU values of the dietary protein source

Diet	description	Live-weight (g/chick) 1-week	Live-weight (g/chick) 3-week	Intake (g/chick) Food	Intake (g/chick) N	% of carcass H_2O	% of carcass N	NPU
				Experiment 1				
8A	Protein-free diet	71.6±0.3	55.3±0.5	70	0.03	67.5±0.5	2.48 ±0.02	-
8B	Guar meal (GM-1)	71.7±0.2	64.1±0.5	84	1.83	75.9±0.2	2.37 ±0.02	9.8±1.2
				Experiment 2				
9A	Protein-free diet	70.9±0.2	52.9±0.7	64	0.02	70.3±0.5	2.60±0.02	-
9B	Water extracted-GM-1	70.6±0.2	54.9±1.7	48	0.95	78.3±0.5	2.24±0.04	-
9C	Toasted-GM-1	71.1±0.4	63.4±0.7	85	1.76	76.4±0.3	2.42±0.02	10.2±1.0
9D	Autoclaved-GM-1	70.7±0.2	70.4±0.9	113	2.41	74.5±0.8	2.39±0.03	13.7±1.2
9E	D + 1% L-Lysine	70.9±0.1	66.0±0.5	97	2.18	76.5±0.5	2.39±0.01	10.6±1.0
9F	D + 0.75% DL-Methionine	71.0±0.2	71.1±1.3	105	2.27	75.7±0.2	2.56±0.03	20.6±0.3
9G	D + 1% L-Lysine + 0.75% DL-Methionine	70.8±0.1	69.5±0.4	95	2.19	77.3±0.5	2.58±0.01	20.2±0.7

Values are means ± SE for quadruplicate groups of five male chicks.

period. Toasting the meal (9C) did not appreciably improve the NPU value or the 3 week body weight of chicks from those fed the commercial guar meal (8B) in experiment 1. Autoclaving the meal (9D), however, raised the NPU value to 13.7. Chicks fed the diet containing autoclaved meal (9D) consumed more food and nearly, though not quite, maintained their body weight during the 2 week feeding period.

Addition of 1% lysine to a diet containing autoclaved guar meal (9E) was not found beneficial, but methionine at 0.75% dietary inclusion level (9F) raised the NPU value significantly ($P<0.05$). The inability of lysine supplementation to improve the NPU was again observed when its addition to a diet containing 0.75% methionine (9G) did not change the NPU value.

6.4 NPU experiment 3

Object In the previous experiment described under 6.3, both autoclaving of guar meal and supplementation of methionine to guar meal-based diets were found to improve the NPU of the meal for chicks. It was, therefore, considered desirable to re-investigate their effect in greater detail.

The object of this experiment was to evaluate samples of commercial guar meal (GM-1) after autoclaving at 121°C for varying periods of time. The response of autoclaved meal to graded amounts of added methionine was also studied. In addition a sample of soya bean meal was simultaneously evaluated to act as a positive control.

Birds and management 180 one-week old Ross I male broiler chicks were randomly distributed in groups of five in 36 separate cages located in a house under controlled environment. The rearing of chicks during the first week of age, their selection, randomisation to the cages, and management during the feeding period were the same as described under 6.2. Live body weights and food intake per pen were recorded.

Diets There were nine diets, 10A to 10I. Diet 10A was a protein-free control diet, the composition of which is given in table 6.1. Diet 10B contained 29% soya bean meal substituted for glucose in the control diet. Diets 10C to 10G each contained 35% GM-1 which had been autoclaved for 10, 20, 30,40 and 50 min respectively. Diets 10H and 10I were similar to diet 10E but contained 0.5 and 1% supplemental DL-methionine respectively. Because the protein sources were incorporated in diets at a fixed level the dietary crude protein contents were not exactly 13% but around 13%. All diets were offered ad lib. for a 2-week period to quadruplicate groups of five chicks. Guar meal samples were autoclaved at 121°C

for definite periods of time as described earlier (Expt. 4.2).

Analysis At the end of the feeding period all the chicks were weighed individually, killed, and processed pen-wise according to the procedure given in 6.2. The carcass nitrogen contents of the chicks in the respective dietary groups and their nitrogen intakes were established.

Results Data on live-weights, food and nitrogen intakes, body-water and -nitrogen contents of chicks and the NPU values of the dietary protein-source are set out in table 6.3.

It can be seen that chicks in all the dietary groups except those fed on the protein-free diet (10A) gained weight during the 2-week feeding period. Chicks fed the diet containing soya bean meal (10B) had significantly higher ($P<0.001$) 3-week weights and consumed significantly more ($P<0.001$) food than those fed on diets containing guar meal (10C to 10I). A NPU value of 54.2 ± 0.5 was obtained for soya bean meal used in this experiment.

Chicks fed on diets containing autoclaved guar meal (10C to 10I) had almost similar 3-week body weights. Increasing the autoclaving time from 10 to 40 min (10C to 10F) did not appear to have any significant effect on the NPU value, although a numerically higher value of 18.5 ± 1.5 was obtained for meal autoclaved for 10 min. An autoclaving time of 50 min. at $121^{\circ}C$, however, resulted in a very low NPU value of 4.4 ± 0.9 only. Guar meal autoclaved for 30 min (10E) gave a NPU value of 15.3 ± 1.8 which compares favourably to the value of 13.7 ± 1.2 obtained in a previous experiment (Table 6.2) for a similarly processed guar meal sample.

Addition of methionine at 0.5% level in diet containing guar meal which was autoclaved for 30 min (10H) significantly improved

Table 6.3

Live-weights, food and nitrogen intakes, body-water and -nitrogen contents of chicks and the NPU value of the dietary protein source

Diet/description		Live-weight (g/chick)		Consumption (g/chick)		% of carcass		NPU
		1-week	3-week	Food	N	H_2O	N	
10A	Protein-free diet	74.9±0.9	57.4±0.4	105	0.04	68.6±0.9	2.40±0.03	-
10B	Soya bean meal	75.4±1.2	160.0±4.2	271	5.49	68.2±0.2	2.69±0.01	54.2±0.5
10C	GM-1, autoclaved, 10 min.	76.6±2.6	81.0±2.9	162	3.65	72.5±0.5	2.47±0.02	18.5±1.5
10D	GM-1, autoclaved, 20 min.	76.2±1.5	82.5±1.8	172	4.01	73.6±0.8	2.39±0.03	15.9±1.1
10E	GM-1, autoclaved, 30 min.	76.3±2.8	82.7±4.3	160	3.67	72.7±0.5	2.31±0.01	15.3±1.8
10F	GM-1, autoclaved, 40 min.	74.6±1.9	85.1±1.5	179	3.98	71.4±0.3	2.37±0.01	17.2±0.3
10G	GM-1, autoclaved, 50 min.	76.5±2.5	85.5±2.5	196	4.33	71.6±0.4	1.79±0.01	4.4±0.9
10H	Diet E + 0.5% methionine	73.5±1.9	84.6±2.2	177	3.90	72.5±0.5	2.46±0.02	19.1±0.4
10I	Diet E + 1.0% methionine	74.5±2.4	85.0±4.2	170	3.73	73.3±0.7	2.59±0.02	23.1±1.4

Values are means ± SE for quadruplicate groups of five male chicks.

($P<0.05$) the NPU value when compared to the value obtained with similarly autoclaved but methionine-unsupplemented guar meal (10E). Increasing the level of added methionine to 1% dietary level (10I) resulted in a further significant ($P<0.05$) improvement in the NPU value of autoclaved guar meal for the growing chicks.

6.5 NPU experiment 4

Object This experiment was conducted to evaluate the protein quality of another sample of commercial guar meal (GM-3) both before and after processing. The effects of methionine and of enzyme supplementation on its protein utilisation by the chicks were also studied.

The three laboratory-processed guar meal samples used in this experiment included sodium hydroxide-extracted, ethanol-extracted and autolysed guar meal. The two enzyme preparations used, MKC hemicellulase and Bromelain 1100 (protease), were obtained from Miles Kali-Chemie GmbH & Co.

Extraction with sodium hydroxide solution A known amount of guar meal was added to 5 volumes of 0.1% NaOH solution. The material was mixed well and then allowed to stand for 15 min, after which time the supernatant liquid (tan to reddish in colour) was decanted. The process was repeated with another five volumes of sodium hydroxide solution. The residual material was washed free from alkali by washing five times with tap water, using five volumes of water each time. The washed material was spread in layers of about 16mm on to the aluminium foil-lined metal trays and dried overnight in a forced-draft hot-air oven at 85°C. The yield (air dry basis) was approximately 53% of the fresh material extracted. The dried product was ground to a fine meal before incorporating in the experimental diets. The chemical composition of the processed meal is set out in Appendix B 6.

Extraction with ethanol About 800g of guar meal was transferred into a large thimble and extracted with approximately 3 litres of boiling 80% ethanol for 24 hours using a soxhlet extraction apparatus. The temperature inside the soxhlet flask remained around 50°C during the period of extraction. Following extraction the material was dried in a forced-draft hot-air oven at 50°C before incorpor-

ating in the diets. The loss of material as a result of the extraction was about 6%. Chemical composition of the extracted meal is given in Appendix B6.

Autolysis of guar meal The required amount of guar meal and water (80 : 20, w/v) were transferred to a HOBART dough mixer. After mixing for 30 min the material was spread on to the aluminium foil-lined metal trays in layers of about 20mm thick and allowed to stand at room temperature for 6 hours. At the end of this time the trays were placed in a forced-draft hot-air oven at 107°C for 120 min. Immediately after heating the meal was removed from the oven and transferred to other trays where it was spread in layers of about 20mm and left overnight at room temperature.

Birds and management 160, one-week old Ross I male broiler chicks were randomised to groups of five and housed in separate compartments of thermostatically-controlled battery brooders. Four groups were randomly assigned to receive one of eight experimental diets for a 2-week period. Food and drinking water were available at all times. Chicks were weighed individually before and after the feeding period and food intake per pen were recorded.

Diets There were eight experimental diets; 11A to 11H. Diet 11A acted as the protein-free control, the composition of which is set out in table 6.1. Diets 11B to 11E each contained commercial guar meal (GM-3) and diets 11F, 11G and 11H contained sodium hydroxide-extracted, ethanol-extracted and autolysed guar meal respectively. Diet 11D was supplemented with 0.1% MKC hemicellulase and diet 11E with both, 0.1% MKC hemicellulase and 0.02% Bromelain 1100. All diets except 11A and 11B also contained 0.5% added methionine.

Analysis At the end of the feeding period chicks in respective groups were weighed, sacrificed and processed (6.2) and the total carcass nitrogen determined.

Results The live-weights, food and nitrogen intakes and body-water and -nitrogen contents of chicks fed on the various experimental diets along with the NPU values of the test proteins are given in Table 6.4.

Chicks fed on the diet containing GM-3 (11B), as the only source of protein, nearly, though not quite, maintained body-weights and the diet was calculated to have a NPU value of 17.5±1.2. When this diet was supplemented with 0.5% methionine (11C), chicks, despite eating numerically less food, showed positive weight-gains and the NPU Value rose to 20.1. Weight gain, food intake and NPU value increased further with the addition of 0.1% MKC hemicellulase in the diet (11D). Addition of 0.02% Bromelain 1100 (11E) however did not result in any increase in the performance of chicks compared to those fed on diet 11D. Inclusion of sodium hydroxide-extracted guar meal in the diet (11F) resulted in the best performance by chicks and gave the highest NPU value (40.5). A significant increase in weight gain, food intake and NPU value was also observed among chicks fed on the diet containing ethanol-extracted guar meal (11G). Autolysing the meal (11H), however, resulted in no improvement in its utilisation by the chick.

There was no mortality during the experiment.

Table 6.4

Live-weight, food and nitrogen intakes, body-water and -nitrogen contents of chicks and NPU values of dietary protein sources

Diet/description		Live-weight (g/chick)		Consumption (g/chick)		% of carcass		NPU
		1-week	3-week	Food	N	H_2O	N	
				Experiment 4				
11A	Protein-free diet	72.8±0.5	53.8±1.0	70	0.03	68.3±0.6	2.81±0.03	-
11B	Guar meal (GM-3)	72.8±0.5	71.8±2.3	115	2.50	73.1±0.7	2.68±0.03	17.5±1.4
11C	B + 0.5% DL-Methionine	72.5±0.6	74.8±0.9	101	2.22	74.6±0.2	2.58±0.01	20.1±1.5
11D	C + 0.1% MKC. hemicellulase	72.5±0.6	87.5±3.5	131	2.92	71.4±0.3	2.61±0.01	27.3±1.1
11E	D + 0.02% Bromelain	72.8±0.5	86.3±2.1	127	2.67	72.2±0.6	2.52±0.02	25.8±1.7
11F	NaOH-extracted GM-3 + 0.5% DL-Methionine	72.8±0.5	126.5±4.4	206	4.42	70.0±0.4	2.59±0.01	40.5±0.9
11G	Ethanol-extracted GM-3 + 0.5% DL-Methionine	72.8±0.5	87.0±2.8	129	2.83	71.2±0.5	2.64±0.02	28.9±0.8
11H	Autolysed-GM-3 + 0.5% DL-Methionine	72.5±0.2	73.3±2.5	106	2.22	73.5±0.5	2.52±0.02	16.0±1.3

Values are mean ± SE of quadruplicate groups of 5 male chicks.

6.6 NPU Experiment 5

Object In a previous experiment described under 6.5, supplementation of MKC hemicellulase in a diet containing guar meal was found to have a beneficial effect on its utilisation by the chicks. Because the enzyme was added at one dietary level only it was considered desirable to investigate its effect more fully at several dietary levels. An attempt was also made to look into deficiencies in the guar meal protein of amino acids other than methionine.

Birds and management 180, one-week old male broiler chicks (Marshall's) were randomly distributed to groups of five and housed in separate cages located in one room under a controlled environment. Rearing of the chicks during the first-week of life, selection, randomisation to cages, and management during the experimental period were the same as described previously (6.2).

Diets Diet 12A was a protein-free control diet, the composition of which is set out in Table 6.1. Eight test diets (12B to 12I) were prepared by substituting guar meal (GM-3) for glucose in diet 12A, so as to supply approximately 13% protein. MKC hemicellulase at 0.1, 0.2, 0.3, 0.4, 0.5, 0.75 and 1.0% levels was added to diets 12B to 12H respectively. Diet 12I, in addition to 1.0% MKC hemicellulase, also contained 0.2% each of L-histidine, L-isoleucine, L-leucine, L-threonine, and L-tryptophan. Methionine at 0.5% level was added to all guar meal-containing diets (12B to 12I). All diets were offered ad lib. for a 2-week period to quadruplicate groups of five chicks. Food consumption per pen was recorded.

Analysis At the end of the experiment all the chicks were weighed individually, killed and processed according to the general procedure described earlier (6.2). Carcass-nitrogen contents of chicks in the respective dietary groups were established.

Results Data on body weights, food and nitrogen intakes of chicks and the carcass-water and -nitrogen contents together with NPU values of dietary proteins are summarised in table 6.5.

Guar meal (GM-3) when supplemented with 0.5% methionine plus 0.1% MKC hemicellulase (12B), was found to have a NPU value of 28.7±0.5; this confirms the findings in a previous experiment (6.5) where a NPU value of 27.3±1.1 was obtained with a similar dietary treatment, despite the differences in the strains of the chicks used in the two experiments, initial body weights, body-weight gains and the food intakes. The closeness of the two NPU values is of special significance and emphasises the usefulness and precision of the NPU method of protein quality assay.

Data on the 3-week body weights and food intake of the chicks fed on the diets supplemented with increasing levels of MKC hemicellulase (12B to 12H), suggest that there is very little or no advantage of adding enzyme at levels higher than 0.2% (12C). Also, increasing the level of enzyme in the diet had no appreciable effect on the NPU value of guar meal. Chicks fed on the diet supplemented with the amino acid mixture (12I) exhibited better performance and a higher NPU value was obtained than those fed on diets containing no added amino acids other than methionine.

There was no mortality during the experimental period.

Table 6.5

Live-weights, food and nitrogen intakes, body-water and -nitrogen contents of chicks and the NPU value of the dietary protein source

	Diet/description	Live-weight (g/chick)		Consumption (g/chick)		% of carcass		NPU
		1-week	3-week	Food	N	H_2O	N	
12A	Protein-free diet	82.2±0.3	62.5±0.8	118	0.04	65.7±0.3	2.57±0.01	-
12B	GM-3 + 0.5% Meth. + 0.1% MKC hc.*	82.8±0.2	105.1±2.5	199	4.19	70.5±0.5	2.64±0.02	28.7±0.5
12C	GM-3 + 0.5% Meth. + 0.2% MKC hc.	82.2±0.2	100.0±3.0	176	3.71	70.7±0.2	2.72±0.01	30.9±0.6
12D	GM-3 + 0.5% Meth. + 0.3% MKC hc.	82.6±0.3	107.0±2.0	204	4.29	70.0±0.5	2.68±0.02	30.2±0.3
12E	GM-3 + 0.5% Meth. + 0.4% MKC hc.	82.2±0.5	115.1±1.7	219	4.66	69.0±0.1	2.67±0.005	32.1±0.6
12F	GM-3 + 0.5% Meth. + 0.5% MKC hc.	82.7±0.5	108.2±3.9	194	4.13	70.6±0.1	2.63±0.003	30.7±1.0
12G	GM-3 + 0.5% Meth. + 0.75% MKC hc.	82.4±0.1	103.8±2.7	188	3.99	70.2±0.5	2.59±0.02	28.0±0.5
12H	GM-3 + 0.5% Meth. + 1.0% MKC hc.	82.8±0.3	105.1±2.6	194	4.12	70.1±0.4	2.67±0.02	29.2±0.8
12I	Diet H + amino acids**	82.6±0.2	119.4±2.6	205	4.64	71.6±0.4	2.64±0.01	34.2±1.0

* hc. = hemicellulase.

** Contained 0.2% each of L-histidine, L-isoleucine, L-leucine, L-threonine, and L-tryptophan.

Values are means ± SE for quadruplicate groups of five male chicks.

6.7 NPU Experiment 6

Object In the experiments reported in 6.5 and 6.6 supplementation of guar meal-containing diets with MKC hemicellulase was found to have a beneficial effect on the utilisation of protein by the chicks. It was also observed that increasing the enzyme concentration in the diet above 0.2% did not improve the NPU value further. Because the enzyme was added to the diet in a dry form and in view of the short period that food takes to pass through the digestive tract of the chicken, it is possible that its action on the guar polysaccharides may not have been completed. To test this hypothesis guar meal was evaluated after incubation with and without added enzymes. Also the effect of inclusion of cholesterol in diet on the utilisation of guar meal by chicks was studied.

Preparation of autolysed and enzyme-reacted guar meal The required amount of guar meal and water (50:50, w/w) were mixed for 10 min in a HOBART dough mixer. The mixture was then spread, in layers of about 20mm, on to aluminium foil-lined metal trays and incubated at 40°C in a forced-air oven for a period of 48hr. After incubation the material was dried in a forced-draft hot-air oven to about 90% dry matter and ground finely before incorporating into diets.

The enzyme-reacted guar meal was processed similarly as described above except that the necessary amount of enzyme was dissolved in water before mixing. The two enzymes used were Rhozyme HP-150 and MKC hemicellulase.

Birds and management 180, one-week old male broiler chicks (Marshall's) were randomly distributed into 36 groups of five so that each group received one of nine diets for a 2-week period. The groups were placed in separate cages all located in one room under a controlled-environment. Their feeding and management during the first week of

life, selection, and randomisation to the cages were the same as described in 6.2. The chicks were weighed before and after the feeding period. Food and water were available ad lib. at all times.

Diets Diet 13A acted as the protein-free control diet, the composition of which is given in table 6.1. Diet 13B contained a commercial guar meal (GM-2) from the second consignment, and diet 13C contained autolysed GM-3; each of the guar meal samples was substituted for equal parts of glucose in the control diet (13A) so as to provide approximately 13% protein. Diets 13D, 13E and 13F contained GM-3 which had been reacted with 0.1, 0.2 and 0.4% Rhozyme HP-150 respectively. Diet 13G was similar to diet 13F except that the enzyme was not reacted with the meal, but added during mixing. Diet 13H and 13I both contained GM-3 which had been reacted with 1% MKC hemicellulase. Furthermore, 2% cholesterol was added to diet 13H. All the test diets were supplemented with 0.5% of methionine.

Analysis At the end of the experiment chicks in all groups were weighed, killed and processed according to details described earlier (6.2) and their body-nitrogen contents established.

Results Results of the experiment are summarised in table 6.6.

From the data it can be seen that the second sample of commercial guar meal (GM-2) when supplemented with 0.5% dietary methionine (13B) gave a NPU value of 26.3±1.2, significantly higher ($P<0.05$) than the value of 20.1±1.4 obtained in an earlier experiment (6.5) for GM-3 plus 0.5% methionine. The diet containing autolysed GM-3 with 0.5% added methionine (13C) gave a NPU value of 27.4; this suggests that autolysing the meal at 40°C for 48hr after mixing with an equal weight of water is beneficial. The NPU of GM-3 increased significantly over that of autolysed meal

Table 6.6

Live-weights, food and nitrogen intakes, body-water and -nitrogen contents of chicks and the NPU values of the dietary protein source

Diet*	/description	live-weight (g/chick)		consumption (g/chick)		% of carcass		NPU
		1-week	3-week	Food	N	H_2O	N	
13A	Protein-free diet	77.4±1.6	60.3±1.5	90	0.03	66.5±0.7	2.52±0.03	-
13B	Guar meal (GM-2)	79.1±1.9	89.2±2.3	143	3.12	72.2±0.2	2.59±0.01	26.3±1.2
13C	Guar meal (GM-3) autolysed	78.7±1.8	92.4±3.4	192	3.67	71.0±0.3	2.69±0.01	27.4±1.2
13D	GM-3 reacted with 0.1% Rhozyme HP-150	76.6±1.0	95.0±1.2	142	3.00	70.7±0.4	2.58±0.02	32.6±1.0
13E	GM-3 reacted with 0.2% Rhozyme HP-150	79.6±1.5	94.8±2.5	135	2.81	69.8±0.1	2.73±0.02	39.0±0.3
13F	GM-3 reacted with 0.4% Rhozyme HP-150	77.3±1.2	93.5±2.7	145	3.07	70.4±0.4	2.59±0.02	30.2±1.2
13G	GM-3 + 0.13% Rhozyme HP-150**	77.6±1.2	102.4±3.8	172	3.61	69.8±0.2	2.66±0.01	34.1±1.1
13H	GM-3 reacted with 1% MKC hemicellulase	78.6±1.1	106.7±4.4	172	3.74	70.6±0.5	2.68±0.02	36.4±1.5
13I	Diet H + 2% cholesterol	76.7±0.8	130.7±4.4	196	4.01	68.8±0.5	2.56±0.02	46.3±0.7

Values are means ± SE for quadruplicate groups of five male chicks

* All diets except 13A contained 0.5% added DL-methionine

** Equivalent to 0.4% of guar meal

when it was treated similarly but in the presence of 0.1% Rhozyme HP-150 (13D). Reacting the meal with 0.2% Rhozyme HP-150 (13E) resulted in a further increase in the NPU for chicks; reacting the meal with 0.4% enzyme (13F), instead of following this trend, surprisingly resulted in a low value of 30.2±1.2. Addition of Rhozyme HP-150 in dry form to a diet containing GM-3 (13G) had a significant effect on its utilisation and a NPU value of 34.1±1.1 was obtained.

GM-3 reacted with 1% MKC hemicellulase and then supplemented with 0.5% methionine in a diet (13H), gave a NPU value of 36.4±1.5, higher than the figure 30.2±0.3 obtained in a previous experiment (6.6) under similar dietary conditions except that the enzyme was added to the diet in a dry form. Addition of 2% cholesterol to the diet containing MKC hemicellulase-reacted GM-3 (13I) gave a highest NPU value of 46.3±0.7 for any guar meal sample, treated or otherwise, used in this series of experiments. The 3-week bodyweight and food intake of chicks fed on this diet were also numerically higher than those fed on other guar meal-containing diets.

6.8 NPU Experiment 7

Object This experiment was carried out to assess the effect of a purified guar gum sample on the utilisation of dietary protein. The only source of protein used was a commercial sample of herring meal which was presumed to be free from toxic constituents such as saponins or mucilaginous materials.

Birds and management One hundred, one-week old Marshall's male broiler chicks were randomly distributed in groups of five and each group housed in separate cages, all located in one room under a controlled environment. The general management, selection and randomisation of chicks were the same as described earlier (6.2).

Diets Diet 14A acted as the protein-free control, the composition of which is set out in table 6.1. Diets 14B to 14E were formulated by substituting herring meal at the expense of glucose in the control diet so that they contained approximately 13% protein. Guar gum (Sigma Chemicals) was added at 0.5, 1.0 and 2.0% levels to diets 14C, 14D and 14E respectively. All the diets were offered ad lib. to quadruplicate lots of five chicks for a 2-week period. Food consumption per pen was recorded.

Analysis At the end of the feeding period all chicks were weighed, killed and processed according to the procedures described earlier (6.2). Total body-nitrogen of chicks were established.

Results The results of the experiment are summarised in Table 6.7.

The commercial sample of herring meal used in this experiment (14B) was found to have a NPU value of 62.0±1.0 for the growing broiler chicks. When supplemented with 0.5% guar gum (14C) the NPU value fell significantly to 50.6. Increasing the dietary level of guar gum to 1.0 and 2.0% (14D and 14E) significantly ($P<0.05$) lowered the NPU values to 43.8 and 39.7 respectively. Chicks fed

Table 6.7

Live-weights, food and nitrogen intakes, body-water and -nitrogen contents of chicks and the NPU values of the dietary protein source

Diet/Description		Live-weight(g/chick)		Consumption(g/chick)		% of carcass		NPU
		1-week	3-week	Food	N	H_2O	N	
14A	Protein-free diet	77.4±1.6	60.2±1.4	90	0.03	66.5±0.7	2.52±0.02	-
14B	Herring meal (HM)	74.8±1.3	176.0±0.2	241	5.08	67.8±0.3	2.64±0.01	62.0±1.0
14C	HM + 0.5% guar gum	79.8±0.8	171.3±12.1	267	5.78	68.3±0.3	2.59±0.01	50.6±1.8
14D	HM + 1.0% guar gum	77.5±1.6	151.9±6.3	260	5.43	67.7±0.3	2.55±0.01	43.8±1.0
14E	HM + 2.0% guar gum	78.7±0.8	132.1±4.4	226	4.83	70.1±0.3	2.58±0.01	39.7±1.9

Values are means ± SE for quadruplicate groups of five male chicks.

diets containing 0.5 and 1.0% guar gum (14C and 14D) consumed more food than those fed on the diet with no added gum (14B); food intake dropped sharply when the level of gum in the diet increased to 2.0% (14E). Inclusion of guar gum in the diet adversely affected the weight gain of chicks, the effect being almost linear. A level of 0.5% gum in diet (14C) reduced the weight gain of chicks by about 9% and those fed on the diet containing 2% guar gum (14E) gained only about 53% body weight to those fed on the control diet (14B).

6.9 Discussion

Of the three commercial guar meal samples assayed, GM-1 was found to have the lowest NPU value for the growing broiler chick. When fed in a diet as the only source of protein at an inclusion level of approximately 35%, the chicks were unable to maintain their body weights. A similar effect has been reported by Nagpal et al. (1971) with chicks fed a diet containing 40% guar meal as the sole protein source.

In the literature surveyed no data on the NPU value of guar meal for the chick appear to have been reported. Although Alvi et al. (1964) found NPU values for rats of 10 and 48.5 for the guar seed flour and the cooked guar germ meal respectively when fed at 10% dietary protein levels. In view of the differences in the amino acid requirements (e.g. Almquist, 1972), tolerance for dietary guar gum (Ershoff and Wells, 1962; Booth et al., 1963) and the protein contents of the assay diets of chicks and rats, differences in the NPU values of the two species might well be expected.

Effect of heat treatment Toasting of GM-1 at 107^{o}C for 2hr did not result in improved utilisation of its protein by the chicks. This finding corroborates the observations of the growth experiment (4.1) where inclusion of 10% dietary toasted-guar meal depressed the performance of chicks to much the same extent as 10% untreated guar meal.

Autoclaving GM-1 at 121^{o}C for periods ranging from 10 to 40min considerably increased its NPU value and the chicks showed positive weight gains. Increasing the autoclaving time to 50min, however, adversely affected the NPU value. This may indicate that the heat was starting to damage the protein. The beneficial effects on NPU of autoclaving GM-1 for short periods suggests the presence

of some heat-labile antinutritional substances in the meal. A sample of this meal gave a positive response to an *in vitro* trypsin inhibitor assay, although the level found was fairly low and was considered to be of doubtful nutritional significance. It is, however, possible that, *in vivo*, the inhibitor reduces the nutritive value of the protein and, if such an inhibitor were heat-labile (Hooper and Couch, 1971) would account for the response to autoclaving. Furthermore this meal was found to have a high microbial population (Table 8.1) and the response in NPU to heating could be a measure of the reduction of the harmful effects of these microflora. Another sample of guar meal (GM-3), which was found negative to a trypsin inhibitor test, gave a NPU value of 17.5±1.2 for the growing broiler chick, indicative of its better quality protein than GM-1.

Extraction with water Feeding of water-extracted GM-1 as the only source of protein in diet was found deleterious to chicks and resulted in about 50% mortality within the 2 week feeding period. The chemical composition of the extracted meal (Appendix B 6), however, shows no significant changes other than the available carbohydrate contents of the meal due to extraction. It was therefore assumed that certain toxic substances were formed or released during the processing. A detrimental effect of water-soaked guar meal on chick growth has also been observed by Kawatra *et al*. (1968).

Effect of amino acid supplementation Reference to Table 6.8 shows that guar meal is fairly deficient in certain essential amino acids although remarkably rich in arginine. In view of the low sulphur-containing amino acid contents and the chick's higher requirement for these amino acids, it is not surprising that supplementation of a guar meal-containing diet with methionine consistently increased the NPU value for the chick. Addition of 0.5, 0.75 or 1.0% methionine

Table 6.8

Ability of guar meal protein to supply the amino acid requirements of the broiler chicks at 20% dietary protein level

	Requirement* (% in diet)	Per cent of chick requirement supplied		
		GM-1	GM-2	GM-3
Arginine	1.20	216	198	186
Glycine/serine	1.00	192	186	185
Histidine	0.40	127	125	121
Isoleucine	0.75	87	85	81
Leucine	1.70	69	68	66
Lysine	1.10	76	73	76
Methionine	0.40	43	72	48
Cystine	0.35	72	79	78
Phenylalanine	0.70	102	106	101
Threonine	0.70	85	89	93
Tryptophan	0.20	76	100	96
Tyrosine	0.60	151	141	139
Valine	0.85	88	73	85

* NRC (1971)

to diets containing autoclaved GM-1 increased the NPU values to 19.1, 20.6 and 23.1 respectively. Likewise, supplementation with 0.5% methionine in a diet containing GM-3 increased its NPU value from 17.5 to 20.1. The higher NPU value obtained for GM-2 supplemented with 0.5% methionine may be a reflection of the greater amount of that amino acid in the meal itself as is seen in Table 6.8.

Addition of lysine alone or in combination with methionine to diets containing GM-1, however, had no effect on the NPU value although reference to Table 6.8 indicates a deficiency of that amino acid in guar meal. The lack of response to lysine supplementation may be due to the low maintenance requirement of the chick for this amino acid (Fisher et al., 1960). Addition of 0.2% each of histidine, isoleucine, leucine, threonine and tryptophan to a diet containing GM-3 increased its NPU value further and the chicks showed greater growth potential. From the amino acid composition (Table 6.8) this positive response would appear to be most likely due to the leucine and/or isoleucine.

Extraction with sodium hydroxide and ethanol After extraction with dilute sodium hydroxide solution, the nutritive value of guar meal for the chick was greatly improved. The extracted product when supplemented with 0.5% dietary methionine gave a NPU of 40.5±0.9, a value which is twice as much as that of unextracted GM-3 and is even higher than the NPU values of linseed meal, cottonseed meal, safflower meal, isolated peanut protein, feather meal and meat meal reported by Fisher (1973). The food intake and weight gain of chicks also increased significantly when fed on the diet containing sodium hydroxide-extracted guar meal. The principal drawback in the sodium hydroxide extraction lay in the substantial loss of material. From the proximate chemical composition of the extracted meal

(Appendix B 6) the low yield appeared to be mainly as a result of loss of meal as such rather than any specific component. However the amino acid pattern of the meal, although generally fairly similar to that of the unextracted meal (Table 2.7), differs importantly in having a considerably higher methionine content. It appears that protein lost during alkali treatment contains relatively small quantities of methionine. The increase in methionine level offers an explanation for at least part of the improvement observed in NPU.

To explore the possibility that one of the toxic factors in guar meal could be a saponin(s), the meal was extracted with 80% ethanol. The ethanol-extracted meal was found to have a NPU of 28.9±0.8, a value which is significantly higher than that obtained for unextracted meal. There was also an increase in the food intake and weight gains of chicks fed on the diet containing the ethanol-extracted meal. Since it is most unlikely that 80% ethanol will extract the gum from the meal this experiment provides supporting evidence for the presence of an ethanol-soluble toxin, which could be a glycoside such as saponin. Further indirect support for this hypothesis came from the favourable effect that supplementation of dietary cholesterol had on the NPU of a guar meal-containing diet. Cholesterol has been reported to form stable complexes with saponins (Newman et al., 1958; Griminger and Fisher, 1958) although the chemistry and nutritional consequences of this reaction are not yet fully understood (Birk, 1969; Cheek, 1976).

Effect of enzymes The adverse effects of dietary guar gum on the performance of chicks and on the NPU of herring meal (Figure 6.1) are findings of considerable nutritional importance. They support the observations of Kratzer et al. (1967) who showed that dietary

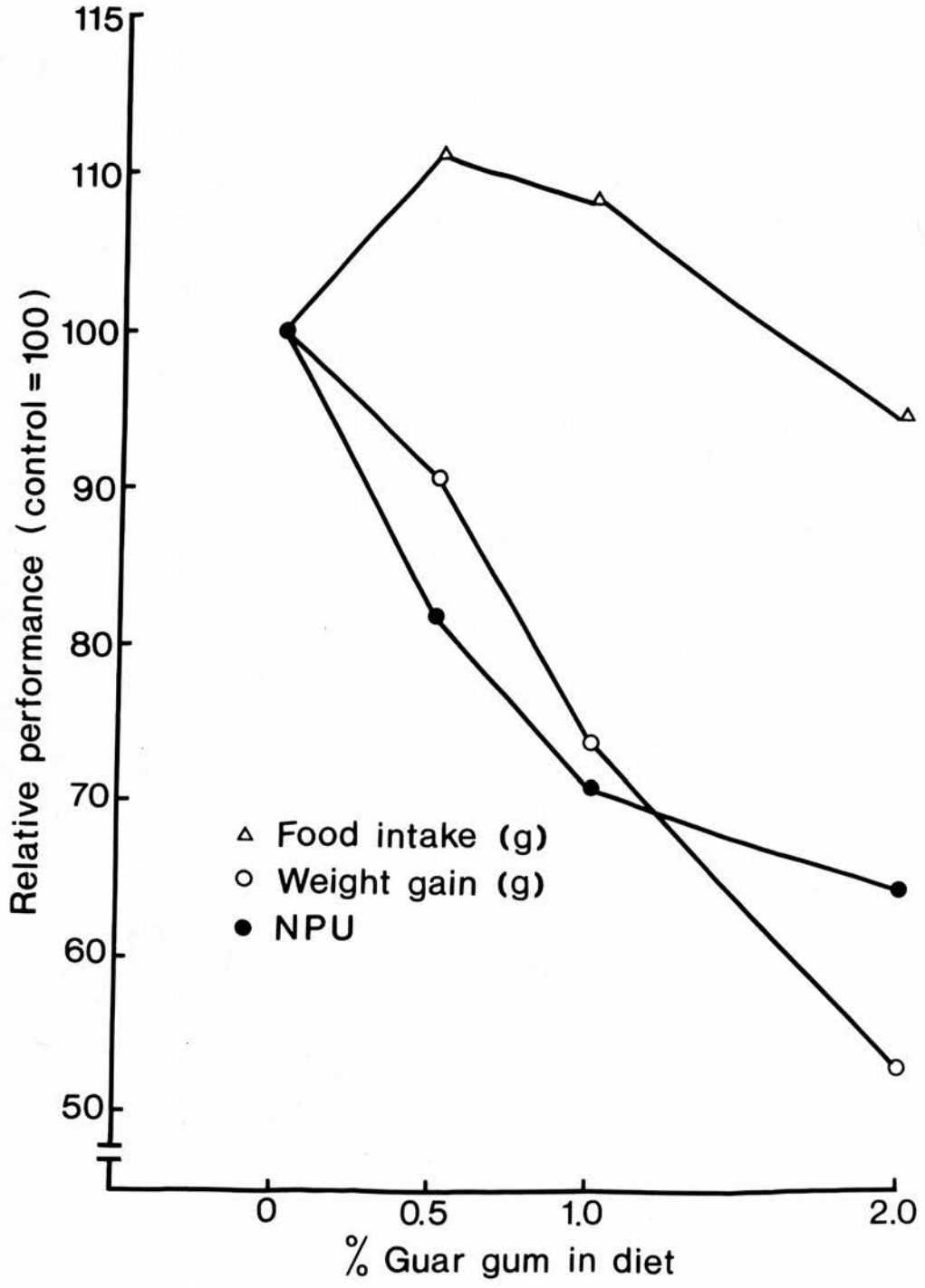


Fig 6.1 Effect of addition of guar gum in diet on the NPU of herring meal and the performance of chicks.

nitrogen retention was lowered when 2% guar gum was included in poultry diets. The low NPU value of guar meal may in part be due to the presence of residual gum in the meal. The increase in the NPU value and weight gain of chicks when fed on guar meal containing-diets supplemented with either of two hemicellulases, MKC hemicellulase and Rhozyme HP-150, strengthens this conclusion.

The higher NPU of GM-3 after autolysing for 48 hours points to the possible presence of endogenous enzymes in the meal capable of hydrolysing the gum and corroborates findings of Sehgal et al. (1973).

The beneficial effect of cholesterol on the NPU value of guar meal is indeed of special significance possibly pointing to the presence of a toxic saponin (Birk, 1969). This effect warrants further investigation.

Conclusions Guar meal appears to have a low quality protein for the broiler chick although it can be greatly improved by supplementation with methionine. The presence of residual gum in the meal adversely affects protein utilisation. This is most likely to be caused by reduced absorption of the nutrients through the intestinal wall due to the gum's property of forming a highly viscous solution with water. Supplementation of diets with enzymes capable of hydrolysing the galactomannan gum, extraction of meal with dilute alkali or ethanol, and addition of cholesterol in diet remarkably improved the utilisation of guar meal protein by the broiler chick.

7. METABOLISABLE ENERGY ASSAY

7.1 Introduction

The concepts of energy for poultry nutrition have been reviewed by many workers (for example, Vohra, 1966; Lockhart, et al., 1967; Kurnick, 1967; Titus and Fritz, 1971). Among the various forms by which the energy value of any feedstuff could be expressed, metabolisable energy (ME) is the most commonly used term for poultry. ME is the difference between the gross energy of the food eaten and the gross energy of the excreta (urine and faeces) and thus represents the total amount of energy supplied by the food which the animal can utilise for its various biological functions. Due to the mixed excretion of faeces and urine in this species, the digestible energy of diets cannot be determined without surgically altering the alimentary tract but, on the other hand, it makes the determination of ME simpler.

ME is a more precise measure than the productive energy, is reproducible, and found to be independent of the plane of food intake in the range of 100 to 30% of ad libitum (Hill and Anderson, 1958).

Recent reviews on the determination of ME of diets and feedstuffs for poultry are available (Vohra, 1972; Miller, 1974). The ME of a diet or feed ingredient can be measured either directly in a bioassay (Hill and Anderson, 1958; Potter et al., 1960) or indirectly from a knowledge of its chemical composition (Carpenter and Clegg, 1956; Sibbald et al., 1963; Bolton, 1962) or from the digestibility data (Fraps, et al., 1940; Titus and Fritz, 1971). The estimation of ME from digestibility data is subject to greater errors; it entails considerably more chemical analysis and assumptions about the heat of combustion of digestible nutrients. The ME values calculated from chemical composition may also suffer from the same drawback. While the indirect methods of ME determinations are quick and less expensive, they do not measure the true response of the animal as is done in a

bioassay and, as such, their use cannot be generalised.

The validity and reproducibility of the ME assays have also been questioned (Kohler and Kuzmicky, 1970; Rao and Clandinin, 1970; Halloran, 1972; Vohra, 1972; Vohra and Kratzer, 1972). Conventional procedures of determining the ME values of diets and feed ingredients for poultry still furnish useful information and are likely to continue until improved or more suitable methods become available.

Although guar meal is a protein-rich feedstuff, the contribution its inclusion in diets makes towards the dietary energy is also of considerable importance. The ME value of guar meal to poultry has not been widely reported and, from the literature surveyed, only one report, by Nagpal et al. (1971), assigns a ME value of guar meal.

As part of the nutritional evaluation of guar meal for poultry, therefore, studies were also carried out to establish its ME value and efforts to enhance the same, by processing the meal or by supplementation with certain enzyme preparations, were also made.

7.2 General procedure

Diets

A diet composed of commonly used poultry feed ingredients was formulated and served as a reference. The test diets were prepared by substituting the ingredient to be assayed for a representative portion of the basal in the reference diet. The individual feed ingredients were ground, if necessary, to pass through a 1.5mm screen before mixing together. The minerals and vitamins in the reference and test diets were added as a supplement. Diets were offered to chicks *ad lib.* as mash.

Collection and processing of droppings

For the determination of ME the total collection method was employed. Birds were housed in individual wire cages located in a controlled-environment house. Enough space was left vacant in between adjacent cages to avoid the possibility of cross-contamination of droppings. Each cage was fitted with an individual feeder, a water cup and plastic droppings tray. Birds were placed on the experimental diets for four days before the balance period, which lasted three or four days. Two hours before the start of the balance period food was removed. During this time food troughs with known amounts of food were prepared and cages cleaned of any clinging droppings. The droppings trays cleaned and fresh water in clean cups was provided. The birds were then offered the experimental diets for a 3 or 4 day period during which droppings were collected once daily. Prior to droppings collection the trays were blown free of down and scales. Particular care was taken to minimise the food spillage. Any spilled food was brushed aside at least twice daily and returned to the food troughs. The droppings from each unit were scraped using a stainless-steel spatula, transferred to the appropriate bags and the bags stored in a

Table 7.1

Per cent composition of the basal and supplement portions for use in the reference diet

Basal	
Maize meal	50
Soya bean meal	30
Herring meal	10
Meal-and-bone meal	5
Maize oil	5
	100
Supplement	
$CaCO_3$	35
$CaHPO_4$	44
NaCl	5
Choline chloride*	4
DL-methionine	2
Mineral premix**	5
Vitamin premix**	5
	100

* Contained 50% choline chloride with silica as base.

** Compositions of mineral and vitamin premixes are set out in Appendix B 1.

deep-freeze at -23°C. The same process was repeated on subsequent days. On the final day food was removed 2 hours before the end of the balance period, weighed and net food intake for each cage established. Any droppings clinging to the cage were scraped and transferred to the appropriate bags. The combined frozen droppings from each bag were transferred on to aluminium dishes of known weights, dried at 80°C for 24 hours in a forced-draft hot-air oven, and weighed. The dried droppings were ground to pass through a 40-mesh sieve and, together with diet samples, analysed for gross energy using a bomb calorimeter and for nitrogen (AOAC, 1965).

Calculation of ME value

The ME value of each diet was calculated from the balance data and corrected to zero nitrogen retention by adding to the excreta energy the energy of the amount of uric acid equivalent to the nitrogen retained per gramme of feed (8.22kcal/g uric acid nitrogen). The calculations were made as follows:

ME_n of diet (kcal/g) = Gross energy of diet (kcal/g) - (Gross energy of droppings (kcal/g) + 8.22 X g N retained/g diet).

Having obtained the ME values of the reference and test diets, the ME value of the test ingredient was derived using the following equation:

$$ME_n \text{ of test ingredient} = \frac{ME_n \text{ of test diet} - (ME_n \text{ of reference diet} \times \frac{\% \text{ of basal in test diet}}{\% \text{ of basal in reference diet}})}{\% \text{ of test ingredient in test diet}} \times 100$$

When deriving the ME value of the test ingredient it was assumed that its presence in the diet did not affect the utilisation of the energy in the basal portion of the diet.

7.3 ME experiment 1

Object

The object of the experiment was to determine the ME values of a commercial sample of guar meal (GM-1) and of the three laboratory processed guar meal samples for poultry. The three laboratory processed meal samples were, autoclaved (121°C for 30 min), toasted (107°C for 2 hours) and water-extracted (as described in 6.3).

Birds and management

48, one-week old Ross I male broiler chicks were distributed to individual metabolism cages according to a randomised plan. The cages were fitted with food and water cups and located in a thermostatically controlled room. Six chicks were randomly assigned to receive one of the eight diets for an eight-day experimental period including a balance period of the final four days during which records of food intake and droppings output were kept.

Diets

Diet 15A, composed of 95 parts of basal and 5 parts of supplement (Table 7.1), served as a reference control. Diets 15B to 15E were similar to diet 15A except that 10, 20, 30 and 60 parts of basal were replaced by equal parts of GM-1 respectively. Diets 15F, 15G and 15H were similarly prepared by substituting 20 parts of autoclaved GM-1, toasted GM-1 and water-extracted GM-1 respectively for the equal parts of basal in diet 15A.

Techniques

The procedures for collection and processing of excreta samples, chemical analysis of excreta and diet samples and the calculation of the ME values of diets and test ingredients were the same as described earlier (7.2).

Results

The results of the balance study are summarised in Table 7.2. Although the aim of the experiment was to determine the ME values of the test ingredients other data normally collected in such a balance study are also included.

It is seen that inclusion of 10, 20, 30 and 60% GM-1 in the diet resulted in a significant and almost linear drop in the food intake of chicks, apparent metabolisability of diet, nitrogen retention and the metabolisability of the dietary energy. At 20% inclusion level GM-1 was surprisingly found to have a negative ME value which of course turned to positive on autoclaving, toasting or water extracting the meal. Inclusion of GM-1 at 10 or 60% dietary level gave higher values of 1167 and 1194kcal/kg respectively than the value of 537 kcal/kg obtained at 30% inclusion level.

Table 7.2

Food intake of chicks, apparent metabolisability of diet, nitrogen retention and energy values of diets containing guar meal

Diet/description	Guar meal in diet (%)	Food DM consumed (g/chick)	Apparent DM metabolisability (%)	Nitrogen retention (%)	ME_n of diet as fed (kcal/kg)	Gross energy metabolised (%)	ME_n of test ingredient, 90% DM (kcal/kg)
15A Reference diet	-	140±3	63.7±0.7	48.7±1.3	2847±25	68.3	-
15B Guar meal (GM-1)	10	124±12	61.3±1.2	45.1±0.5	2664±54	64.1	1167
15C GM-1	20	108±13	50.5±1.3	38.0±1.4	2157±32	51.9	-ve
15D GM-1	30	81±5	48.0±1.5	33.5±1.9	2109±62	50.3	537
15E GM-1	60	53±7	37.2±1.0	12.0±4.8	1765±57	42.3	1194
15F GM-1, autoclaved	20	108±6	53.5±1.5	42.9±2.3	2273±51	54.4	127
15G GM-1, toasted	20	90±6	49.1±1.4	21.9±2.5	2195±59	51.4	263
15H GM-1, water-extracted	20	80±6	54.3±3.6	39.3±5.8	2392±139	55.7	722

Mean ± SE of six observations per dietary group

7.4 ME experiment 2

Object

In a previous experiment (7.3) very surprising results were obtained when a sample of guar meal was assayed for its ME value for poultry. The object of the present experiment was, therefore, to determine the ME values of the two guar meal samples, GM-2 and GM-3, which were being evaluated for their nutritive value for poultry. Each of the two meal samples were assayed at two inclusion levels, 20 and 40% in diet.

Birds and management

30, one-week old male Ross I broiler chicks were distributed to individual metabolism cages according to a randomised plan. Six chicks were randomly assigned to receive one of five experimental diets *ad lib*. for 8-day experimental period including the final four days of balance period.

Diets

Diet 16A was a reference control and composed of 95 parts of the basal and 5 parts of supplement (Table 7.2). Diets 16B and 16C were formulated to contain 20 and 40 parts of GM-2 respectively at the cost of equal parts of basal in the reference diet. Diets 16D and 16E were identical to diets 16B and 16E respectively except that they contained GM-3 instead.

Techniques

The general techniques used in carrying out the experiment and for computation of the ME values of diets and test ingredients were the same as described earlier (7.2).

Results

Table 7.3 shows the summary of results obtained in the balance trial. The nitrogen corrected ME values obtained for the two guar meal

Table 7.3

Food intake of chicks, apparent metabolisability of diet, nitrogen retention and the energy values of diets and test ingredients

		Guar meal in diet (%)	Food DM consumed (g/chick)	Apparent DM metabolisability (%)	Nitrogen retention (%)	ME_n of diet as fed (kcal/kg)	Gross energy metabolised (%)	ME_n of test ingredient, 90% DM (kcal/kg)
16A	Reference diet	-	143±5	62.2±0.5	51.0±1.0	2671±30	64.8	-
16B	Guar meal (GM-2)	20	152±9	51.2±0.9	40.4±1.1	2232±56	53.4	490))619
16C	Guar meal (GM-2)	40	134±11	41.3±0.7	29.9±1.1	1892±32	44.8	746)
16D	Guar meal (GM-3)	20	135±12	50.7±0.8	38.0±1.0	2288±53	54.4	778))795
16E	Guar meal (GM-3)	40	106±7	42.5±1.7	29.9±1.8	1918±69	45.3	812)

Mean ± SE of six observations per dietary treatment

samples assayed, GM-2 and GM-3 were found to be 619 and 795kcal/kg respectively, the numerically higher ME value obtained for GM-3. A higher ME value was obtained at the higher inclusion rate for each of the two meal samples. The apparent metabolisability of diet dry matter, nitrogen retention and the ME_n of diets containing either of the two guar meal samples were significantly lower ($P<0.01$) than those of the reference diet but did not differ significantly between the two meals at the appropriate level of inclusion.

7.5 ME experiment 3

From the results obtained in the previous ME assays (7.3 and 7.4) it is becoming clear that guar meal despite its high gross energy value (about 4300kcal/kg) has a very low ME for poultry. Experience from broiler growth experiments as well as in the previous ME assays have shown that inclusion of guar meal in chick diet causes sticky droppings which adhere to the wire-screen floor and around the vent of the bird. It was being recognised that this characteristic of guar meal is probably due to the presence in the meal of the gum which apparently appears to have not been metabolised by the chicken.

Object

The object of the present experiment was, therefore, to study the effect of inclusion in diets of two commercially available enzyme preparations, MKC hemicellulase and Betaganase M, which were known to hydrolyse similar gums and also have proved beneficial in a broiler experiment (4.3), on the ME value of guar meal.

Birds and management

24, 10-day old male broiler chicks (Marshall's) were distributed to individual cages according to a randomised plan. Six chicks were then randomly assigned to receive one of four experimental diets for a period of seven days including the balance period of last three days.

Diets

There were four experimental diets. Diet 17A was a reference control and consisted of 95.24 parts of basal and 4.76 parts of the supplement (Table 7.1). Diet 17B was formulated by substituting 38.1 parts of GM-3 for equal amount of basal in the control diet. Diets 17C and 17D were similar to diet 17B but supplemented with 0.1% MKC hemicellulase and 0.02% Betaganase M. All the diets were offered to

chicks as mash ad libitum.

Technique

The procedure followed for the determination of ME was the same as described in 7.2.

Results

Table 7.4 shows the summary of results obtained in the balance trial. It is seen that addition of the two enzymes in diets containing guar meal had beneficial effects on the food intake of chicks, apparent digestibility of diet dry matter, nitrogen retention and the ME values of diets. Although in this trial the ME of GM-3 came out to be considerably higher than the value of about 800kcal/kg obtained in a previous assay (7.4), supplementation in diet of the two enzymes, MKC hemicellulase and Betaganase M increased the ME by 34.5% and 17.6% respectively.

Table 7.4

Effect of enzyme supplementation in diet on food intake of chicks, apparent metabolisability of diet, nitrogen retention and the energy values of diets containing guar meal

Diet/description	Food DM consumed (g/chick)	Apparent DM metabolisability (%)	Nitrogen retention (%)	ME_n of diet as fed (kcal/kg)	Gross energy metabolised (%)	ME_n of ingredient, 90% DM (kcal/kg)	Change in ME_n due to enzyme (%)
17A Reference diet	91±6	64.6±0.9	52.7±1.1	2685±61	65.0	-	-
17B Guar meal (GM-3)	68±4	45.8±0.8	34.4±1.5	2015±48	47.6	1036	-
17C Diet B + 0.1% MKC hemicellulase	83±10	50.4±1.4	38.9±1.4	2154±66	51.0	1393	+ 34.5
17D Diet B + 0.02% Betaganase M	76±5	47.9±0.9	39.0±0.9	2085±37	49.3	1218	+ 17.6

Mean ± SE of six observations per dietary treatment.

7.6 ME experiment 4

Extraction of guar meal with 0.1% sodium hydroxide solution or 80% ethanol was found to have definite beneficial effectson its utilisation by chicks in a protein quality assay (6.5). If those findings were valid then the beneficial effect of processing the meal should as well be reflected in its increased ME value for poultry.

Object

The object of the experiment was to determine the ME values of sodium hydroxide-extracted and ethanol-extracted guar meal samples for poultry. The details of processing the meal are described in 6.5 and their analysis in Appendix B6.

Birds and management

18, one-week old male broiler chicks (Ross I) were distributed to individual cages according to a randomised plan. Six chicks were randomly assigned to receive one of three experimental diets for a eight-day period including the balance period of last four days.

Diets

A diet composed of commonly used poultry feed ingredients was formulated (Table 7.5) and served as a reference control (18A). Diets 18B and 18C were prepared by substituting 40 parts each of the two test ingredients, sodium hydroxide-extracted GM-3 and ethanol-extracted GM-3, for the equal representative portion in the reference diet respectively. The diets were offered to chicks ad lib. as mash.

Techniques

The general procedure followed in carrying out the experiment, collection and preparation of droppings samples, chemical analyses and computation of ME values were the same as described earlier (7.2).

Table 7.5

Per cent composition of the reference diet

Maize meal	18.0
Soya bean meal	30.0
Herring meal	8.0
Groundnut meal	16.0
Meat-and-bone meal	16.0
Maize oil	4.0
$CaCO_3$	4.5
Ca H PO_4	1.0
Na Cl	0.5
Choline chloride *	0.5
DL-Methionine	0.5
Mineral premix**	0.5
Vitamin premix**	0.5
	100

* Contained 50% choline chloride with silica as base

** Compositions of mineral and vitamin premixes are set out in Appendix B1.

Results

Table 7.6 gives the summary of results obtained in the balance trial.

There were no significant differences in the food intake of chicks fed on different diets. Birds fed on the reference diet (18A) metabolised significantly more ($P<0.05$) energy and retained significantly more ($P<0.05$) nitrogen than those fed on diets containing either of the two meal samples (18B and 18C). Although the performance of chicks fed on diets containing the two guar meal samples did not differ significantly from each other, those fed on diet containing sodium hydroxide-extracted guar meal (18B) performed consistently better. The extracted meal samples were found to have higher ME values than the value obtained for the unextracted GM-3 in a previous bioassay (7.4).

Table 7.6

Food intake of chicks, apparent metabolisability, nitrogen retention and energy values of diets containing sodium hydroxide- and ethanol-extracted guar meal

Diet/description	Food DM consumed (g/chick)	Apparent DM metabolisability (%)	Nitrogen retention (%)	ME_n of diet as fed (kcal/kg)	Gross energy metabolised (%)	ME_n of ingredient 90% DM (kcal/kg)
18A Reference diet	108±8	40.0±1.3	32.0±1.8	2158±78	48.2	-
18B GM-3, sodium hydroxide -extracted	100±4	31.8±1.3	24.8±2.4	1817±61	40.3	1317
18C GM-3, ethanol-extracted	99±12	30.9±1.5	22.5±1.5	1745±64	39.0	1150

Mean ± SE of six observations per dietary treatment

7.7 ME Experiment 5

In a previous ME assay (7.5) addition of the two enzyme preparations in diets containing guar meal appeared to have definite beneficial effects on its utilisation by the chicks and, therefore, strengthened the view that the presence of gum in the meal appears to be the key factor responsible for the low metabolisability of guar meal-containing diets. In the light of this experience and also from other experiments carried out at the same time it was considered necessary to study the usefulness of another enzyme preparation, Rhozyme HP-150, known to be effective against similar gums and mucilages, on the ME value of guar meal for poultry.

Object

This experiment was carried out to study the usefulness of graded amounts of Rhozyme HP-150 on the ME value of a commercial sample of guar meal (GM-3).

Birds and management

Thirty six, four-week old male broiler chicks (Marshall's) of comparable body weights were distributed to individual metabolism cages according to a randomised plan. The chicks were previously reared on a broiler mash (Appendix B4). Five chicks were then randomly assigned to receive one of six experimental diets for a period of seven days including the balance period of last three days during which records of food intake and droppings output for each individual chick were kept. Although the main object of the experiment was to determine the ME values of the diet but data in respect of weight gain, food and water intakes of individual chicks during the feeding period of seven days were collected.

Diets

There were six diets. Diet 19A was a reference diet and

composed of 95.24 parts of basal and 4.76 parts of supplement (Table 7.1). Diet 19B was formulated by substituting 38.1 parts of GM-3 for equal parts of basal in the reference diet. Diets 19C to 19F were all similar to diet 19B except that they were supplemented with 0.05, 0.1, 0.15, and 0.2% Rhozyme HP-150 respectively. The diets were offered to chicks ad lib. as mash. Drinking water was available at all times.

Techniques

The general procedure followed during the experiment in collection, preparation and analyses of the droppings samples and in calculation of ME values was the same as described before (7.2).

Results

Table 7.7 gives the summary of results of the balance trial. Data on mean weight gain, food intake, FCE and water to food ratio of chicks fed on different diets during the 7 day period are set out in Table 7.8. The results were examined statistically, ANOVA Tables are contained in Appendix C4.

Chicks fed on reference diet (19A) performed significantly better ($P < 0.01$) than those fed diets containing GM-3. The performance, in respect of apparent metabolisability of diet dry matter, nitrogen retention, and the gross energy metabolised by chicks fed on diets containing GM-3 with added Rhozyme HP-150 (19C to 19F) was significantly better ($P < 0.05$) than those fed a similar diet with no added enzyme (19B). Addition of Rhozyme HP-150 to diets containing 38.1% GM-3 had no marked effect on the food intake of chicks but significantly improved ($P < 0.01$) its utilization by the chicks. A dietary level of 0.05% Rhozyme HP-150 in a diet containing 38.1% GM-3 appeared to be as good as a higher level of 0.2%.

Table 7.7

Effect of Rhozyme HP-150 on food intake of chicks, apparent metabolisability of diets, nitrogen retention and energy values of diets containing guar meal

Diet/description	Food DM consumed (g/chick)	Apparent DM metabolisability (%)	Nitrogen retention (%)	ME_n of diet (kcal/kg)	Gross energy metabolized (%)	ME_n of test ingredient 90% DM (kcal/kg)	Change in ME_n due to enzyme (%)
19A Reference diet	$249^{a}\pm15$	$66.3^{a}\pm0.5$	$49.7^{a}\pm0.6$	$2910^{a}\pm36$	70.4	-	-
19B Guar meal (GM-3)	$237^{a}\pm 8$	$45.7^{c}\pm0.8$	$29.9^{c}\pm0.8$	$1991^{c}\pm39$	47.4	643	-
19C GM-3 + 0.05% Rhozyme HP-150	$264^{a}\pm25$	$52.2^{b}\pm2.3$	$38.6^{b}\pm2.5$	$2298^{b}\pm74$	54.7	1449	225
19D GM-3 + 0.10% Rhozyme HP-150	$242^{a}\pm36$	$50.0^{b}\pm1.5$	$34.5^{b}\pm1.5$	$2260^{b}\pm77$	53.8	1349	210
19E GM-3 + 0.15% Rhozyme HP-150	$238^{a}\pm 9$	$50.7^{b}\pm2.5$	$34.4^{b}\pm2.4$	$2289^{b}\pm88$	54.5	1425	222
19F GM-3 + 0.20% Rhozyme HP-150	$239^{a}\pm11$	$52.6^{b}\pm2.2$	$34.6^{b}\pm3.0$	$2343^{b}\pm70$	55.8	1567	244

Mean $\pm$ SE of five observations per dietary treatment

Means in the same column bearing the same superscripts are not significantly different ($P < 0.05$)

From the results set out in Table 7.8 it can be seen that birds fed on reference diet (19A) gained significantly more ($P < 0.05$) weight, had significantly better ($P < 0.05$) FCE and consumed least amount of water per unit of food intake than those fed on diets containing guar meal. Although the food intakes of chicks fed on different diets did not differ significantly, those fed on diet 19B had lowest food intake. Addition of Rhozyme HP-150 in guar meal-containing diets significantly improved ($P < 0.05$) the weight gain and FCE of chicks. Increasing the level of enzyme from 0.05 to 0.2% of diet appeared to have no measurable effect on the performance of chicks. Birds fed on diets containing guar meal generally consumed more water per unit of food intake than those fed on reference diet, those fed on diet 19B had highest water consumption/unit of food intake.

Table 7.8

Effect of addition of Rhozyme HP-150 in diets containing guar meal on the performance of chicks

(Experimental period - 7 days)

Diet	Weight gain (g/chick)	Food consumed (g/chick)	FCE* (%)	W/F** ratio
19A	206^{a}	451^{a}	46.4^{a}	174^{a}
19B	81^{c}	388^{a}	20.8^{c}	279^{c}
19C	150^{b}	477^{a}	32.0^{b}	246^{b}
19D	137^{b}	423^{a}	31.5^{b}	259^{c}
19E	144^{b}	424^{a}	34.2^{b}	257^{bc}
19F	134^{b}	410^{a}	32.6^{b}	248^{b}

Each value is a mean of five observations per dietary group.

Values bearing same superscript in the respective columns are not significantly different ($P < 0.05$)

* Weight gain (g)/100g food consumed

** Water intake (g)/100g food consumed

7.8 ME Experiment 6

In an earlier experiment (6.8) it was observed that addition of guar gum to the diet of chicks reduced protein utilisation and retarded their growth. Guar meal, depending on the efficiency of extraction, may contain up to about 18% residual gum. A diet containing 20% guar meal could well be expected to contain about 3.5% gum, an amount which in the light of the results of previous experiments, may have an adverse effect on the utilisation of dietary nutrients by chicks. Whether the adverse effect of guar gum on the utilisation of dietary nutrients is of a general nature or have specificity for one or more nutrients, would be interesting to know.

Object

The object of the experiment was to investigate the effect of addition of guar gum to a practical diet on the utilisation of nutrients by chicks. Because guar gum is a polymer of galactose and mannose (1:2) the effect that the inclusion of a mixture of these monosaccharides in a diet would have on the performance of chicks was also studied.

Birds and management

Seventeen six-week old male broiler chicks (Marshall's FS) were wing-banded, weighed and randomly distributed into individual cages. The cages had raised wire-screen floors, individual droppings trays, food and water troughs and were located in one room under a controlled environment. The chicks were randomly distributed into three groups of five and one of two only and offered the experimental diets ad lib., for a 7-day period including the balance period of final three days. Water was available to chicks at all times.

Diets

A diet identical in composition to diet 3A (Table 4.10) was formulated and served as the basal diet (20A). Diets 20B and 20C were similar to diet 20A except that they contained 2.5 and 5.0% of guar gum (Sigma Chem.) respectively added at the expense of the entire diet. Diet 20D was similar to diet 20C but contained 5.0% of a mixture of D-galactose and D-mannose (35:65) instead. Diets were offered to chicks ad lib. and as mash.

Techniques

The general procedure followed in carrying out the experiment (collection and preparation of droppings samples and calculation of dietary ME values) were the same as described earlier (7.2). In addition to nitrogen and gross energy the diets and droppings samples were also analysed for their amino acid compositions, glucose (Hudson et al., 1976) and ether-extractable oil contents. The oil contents in droppings were determined by acid-ether extraction (Bayley and Lewis, 1965).

Results

Table 7.9 gives the summary of results obtained in the balance trial.

It can be seen that chicks fed on the basal diet (20A) consumed more food than those fed on diets containing guar gum (20B and 20C). The apparent dry matter metabolisability decreased with the inclusion of gum in diet as did the dietary ME. Chicks retained less nitrogen and metabolised lesser amounts of amino acids from the diets containing guar gum than from the basal diet. The mean relative metabolisability of amino acids in presence of 2.5 and 5.0% guar gum in diets were 84.4 and 78.7% respectively, the absorption of arginine was highest and of threonine lowest (Appendix B8).

Table 7.9

Effect of guar gum and a mixture of galactose and mannose on the utilisation of nutrients by chicks[1]

Diet/description	Food DM consumed (g/chick)	Apparent DM metabolisability (%)	Nitrogen retention (%)	ME_n of diet as fed (kcal/kg)	Glucose+Starch digestibility (%)	Apparent fat absorbability (%)	Apparent metabolisability[5] of amino acids (%)
20A Basal[2]	359±12	70.0±0.4	52.0±0.8	2896±17	97.5±0.2	80.3±0.7	80.0±1.0
20B Basal + 2.5% guar gum[3]	305±20	58.0±2.1	42.8±2.3	2266±107	91.0±1.6	40.5±9.0	67.6±1.5
20C Basal+ 5.0% guar gum[3]	264±13	49.6±2.0	35.7±3.2	1967±68	83.2±2.0	25.3±6.5	63.1±1.7
20D Basal + 5.0% Gal + Man[4]	325± 8	70.4±0.1	52.4±0.5	2856±12	97.3±0.01	79.6±0.6	77.4±1.0

1. Five chicks per dietary treatment except in 20D which had only two
2. Identical to diet 3A (Table 4.10)
3. Purchased from Sigma London, Chemical Company Ltd
4. Contained D-Galactose and D-Mannose, 35:65 parts respectively
5. Details provided in Appendix B8.

The digestibility of soluble carbohydrates was adversely affected and the absorbability of fat was considerably lower in the presence of dietary guar gum. The adverse effects on the performance of chicks increased with increasing level of guar gum in diet. The droppings from chicks fed on diets containing guar gum were in general bulky. Two out of five chicks fed on the diet containing 5% guar gum suffered with diarrhoea.

Inclusion in the diet of 5% of a mixture of galactose (1 part) and mannose (2 parts) did not affect the utilisation of dietary nutrients by the chicks.

7.9 Discussion

Assays carried out to establish the ME value of guar meal for poultry have tended to give equivocal results. In the first place, however, it is apparent that guar meal, despite having a high gross energy content of about 4800kcal/kg dry matter has a very low ME for the broiler chicken as determined in a series of bioassays described earlier. The ME value of GM-1 was determined at several inclusion levels and was found to range from a negative value to one as high as 1200kcal/kg (Table 7.2). It is difficult to explain such an unexpectedly high variation in the ME value of the meal. The ME value of 537kcal/kg obtained at 30% dietary inclusion level appears to be the best assessment in view of the ME values of 619 and 795kcal/kg obtained for the other two guar meal samples, GM-2 and GM-3 respectively.

Only one report (Nagpal et al., 1971) has been published with poultry which assigns a ME value to guar meal. These workers found 2622 and 2005kcal/kg ME_n for a commercial sample of guar meal when assayed at 10 and 20% dietary inclusion levels respectively, with 6-week old male chicks. In the same experiment the ME values for autoclaved guar meal were found to be 2069 and 1548kcal/kg at 20 and 40% dietary inclusion levels respectively. These determinations are higher than the values obtained for any of the three commercial guar meal samples assayed during the course of this investigation. However, unpublished data (J.C. Blair, personal communication) from laying hens confirm that the ME value of GM-1 was 900 kcal/kg.

Effect of dietary inclusion level on ME

The results obtained in the present bioassays (Tables 7.2 and 7.3) indicate that the ME value of guar meal is affected by the

dietary level of its inclusion. Although of a different order of magnitude different ME values were obtained at different dietary inclusion levels for guar meal by Nagpal et al. (1971) and substantiate the present findings. In this respect guar meal is unusual because the level of a dietary ingredient is not generally considered to affect its ME value; indeed this assumption is made in the determination and application of ME values to dietary formulations. However the ME of alfalfa (Vohra and Kratzer, 1970) and that of rapeseed meal (Rao and Clandinin, 1970) have both been shown to fall as their dietary levels increased.

Effect of enzyme supplementation on the ME value of guar meal

The effect of dietary supplementation with certain enzyme preparations on the ME value of guar meal was investigated. The three enzymes used, Rhozyme HP-150, MKC hemicellulase, and Betaganase M were chosen for their known (according to the suppliers) ability to hydrolyse gums and mucilages. All three preparations when supplemented in diets containing guar meal (Tables 7.4 and 7.7) increased the utilisation of dietary nutrients by the chicks and, as a result, in each case a higher ME value for guar meal was obtained. These findings also offer an explanation, at least in part, for the improved performance of chicks in the experiments described under 4.3, 4.4, and 6.5 to 6.7 when one or other of these enzyme preparations were added to guar meal-containing diets.

The observations that sodium hydroxide- and ethanol-extracted guar meal samples were utilised better by and found to have higher ME values for the broiler chick than unextracted meal (Table 7.6) provide further proof of the presence of at least one toxic component in guar. Further evidence for the existence of such a factor is provided elsewhere in this thesis (Section 8).

Effect of guar gum on ME values

The data contained in Table 7.9 demonstrate the deleterious effect that guar gum has on energy metabolism by the chick. Thus, although chemical analysis of guar gum by the method of Shannon (1972) reveals that it consists *in vitro* almost totally of components classified in the "available carbohydrate" fraction (R N Foxton, personal communication), *in vivo* it is apparent that the guar gum carbohydrates are not available to the bird. Indeed, far from contributing to the metabolisable energy content of the diet, guar gum appears to reduce the availability of the other nutrients. This finding undoubtedly offers an explanation why, when guar meal - which may well contain appreciable amounts of gum - is included in chick diets, ME values lower than those calculated from the proximate analyses of the dietary ingredients are obtained. It is, therefore, apparent that the application of equations such as that proposed by Bolton (1962) for predicting the ME values of diets or dietary ingredients from a knowledge of their chemical composition must, in certain circumstances, be treated with caution. The detrimental effect that guar gum has on the metabolisability of diets could also explain why the ME value of guar meal appears to fall as its dietary level increases.

8. ASSAYS FOR SOME TOXIC SUBSTANCES IN GUAR MEAL

8.1. Introduction

Efforts to identify the factors responsible for the poor performance of birds fed on diets containing guar meal have been made in several laboratories. The presence of toxic substances such as a heat labile trypsin inhibitor (Hooper and Couch, 1971), haemagglutinins (Tannous and Ullah, 1969) in addition to residual gum contents of up to about 18% (Nagpal et al., 1971) in the meal have been reported. In view of the detrimental effects observed on the performance of birds the guar meal samples were also examined for certain other toxic substances.

8.2 Microbiological and biochemical examination of guar meal

The guar meal samples (GM-1 and GM-2) were examined microbiologically and for the possible presence of mycotoxins (Roberts and Patterson, 1975) at the ADAS laboratory, Shardlow Hall, Shardlow, Derby (A. Hacking, personal communications). The presence of aflatoxins, zearlenone, patulin, trichothene, ochratoxins or citrinin mycotoxins in either of the two meal samples was not detected. Results of microbiological examinations are set out in Table 8.1.

Table 8.1

Results of microbiological examination of the two guar meal samples

		Propagules/g	
		GM-1	GM-2
Actinomycetales	60°C	3300	210
Actinomycetales	40°C	80	-
Moulds*	37°C	290	30
Moulds*	25°C	27000	180
Bacteria		18000	720
Lactobacilli		800	-
Aerobic spore formers		13000	700

* The predominant moulds in GM-1 at 25°C were Mucorales, Aspergillus fumigatus and A. niger, with A. flavus detected at 37°C. GM-2 contained mainly yeasts.

8.3 Assays for tannins and Hydrocyanic acid

The three guar meal samples evaluated during the course of this investigation were analysed for the presence of tannins and hydrocyanic acid (HCN) as per the conventional methods of analysis (AOAC, 1965). Using the titrimetric method used for coffee and tea the presence of tannins in any of the three meal samples was not established. The HCN contents, estimated by the alkaline titration method, of the three meal samples are given in Table 8.2.

8.4 Trypsin inhibitor and haemagglutinins

All the three guar meal samples were tested for their antitryptic activity and for their haemagglutinating activity against chicken blood cells in this laboratory (J.C. Blair, personal communications). The presence or absence of these activities in the meal samples are also shown in Table 8.2.

8.5 Guar gum

Estimation of gum in the guar meal samples was carried out according to the procedure of Whistler and Saarnio (1957). A known amount of sample was extracted with boiling acetone in a Soxhlet apparatus for 8 hours. Following the acetone extraction the sample was extracted with 12 volumes of water at 40^{o}C, pH 6.5, for 16 hours with occasional stirring and then filtered through cheese cloth. The residue was re-extracted similarly with a fresh amount of water and then filtered. The two filtrates were combined together, adjusted to pH 4.5 and centrifuged at 4000 rpm for 30 min to remove precipitated proteinous material. To the centrifugate were added 3 volumes of ethanol with vigorous stirring. The white precipitate of guar gum was removed by centrifugation and vacuum-dried. The estimated gum contents in the three guar meal samples are given in Table 8.2.

Table 8.2

Contents of certain toxic substances in guar meal

Toxic substance	Guar meal sample		
	GM-1	GM-2	GM-3
Tannins	-	-	-
Hydrocyanic acid (mg HCN/100g dry matter)	5.5	13.0	19.2
Trypsin inhibitor	+	+	-
Haemagglutinins	+	-	+
Guar gum (% dry matter)	6.00	4.29	4.14

(-) The presence could not be established

(+) Were found positive

8.6 Isolation of guar meal saponins

A known amount of guar meal was placed in a Soxhlet thimble and extracted with boiling 80% ethanol for 24 hours as described earlier (6.5). The total ethanolic extract was distilled to remove the ethanol. The residue was extracted three times with an approximately equal volume of hot hexane, and the hexane decanted. The extract was then treated, while hot, four times with an approximately equal volume of n-butanol. The total butanol extract was then washed twice with a 5% aqueous sodium chloride solution. The butanol solution was then film evaporated to about 1/3 of the initial volume. The precipitate which formed during evaporation was removed by centrifugation and then washed four times with small amounts of diethyl ether. The yield was approximately 6.0%.

The crude guar meal saponin, as it was tentatively named, thus obtained was a light-brown coloured powder, had a bitter taste and foamed extensively when shaken in water. This material when assayed in this laboratory (J.C. Blair, personal communications) was found to possess strong haemagglutinating activity, comparable to that of a pure saponin preparation (BDH), against the chicken blood cells.

Following these preliminary findings attempts to quantify the saponin contents in guar meal were made. Considerable difficulties in the isolation and quantification of saponin contents in guar meal were experienced. The procedure used was a modification of the Lieberman-Burchard method devised by Gestetner *et al*. (1966). Using soya bean saponins as the standard the saponin contents in guar meal samples were estimated which, together with saponin contents in typical samples of alfalfa meal, are set out in Table 8.3. A scheme for the extraction of saponins from guar meal is given in Figure 8.1.

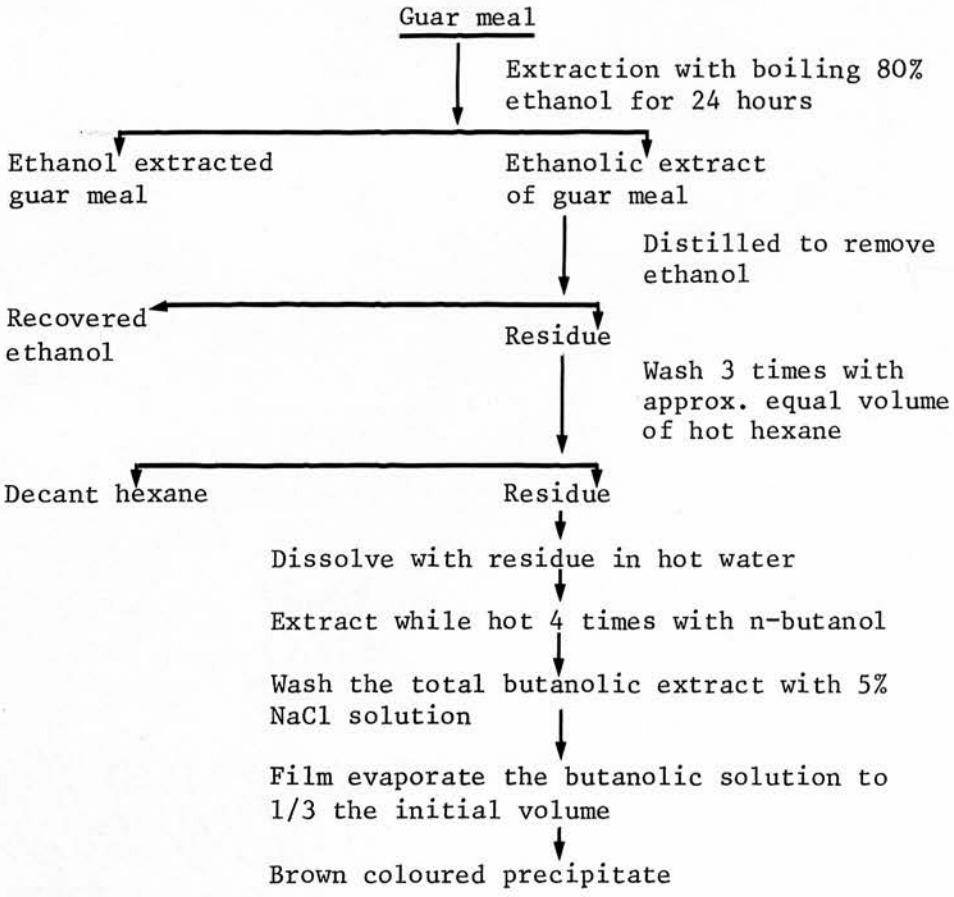
Table 8.3

Saponin content in guar meal and alfalfa meal.

Sample	mg/g dry matter
GM-1	113
GM-2	91
GM-3	136
GM-3, extracted with 80% ethanol	51
GM-3, extracted with 0.1% NaOH	37
Alfalfa meal*	20-30

* Dr C Fisher (Unilever Research, Colworth House, Bedford), personal communications.

Fig. 8.1 Scheme for the extraction of guar meal saponin with ethanol.



Wash four times with diethyl ether and the brown coloured precipitate was removed by centrifugation.

8.7 Discussion and conclusions

The results of assays carried out to test for the presence of certain toxic constituents in the guar meal samples are presented in Tables 8.1, 8.2 and 8.3. The microbiological and biochemical tests, which were carried out only on GM-1 and GM-2, show that the two meals were free from the common fungal toxins. GM-1 did have a higher microbial population than GM-2 and may possibly account for more pronounced undesirable effects observed when this sample was included in chick diets (4.1 and 6.3).

That tannins were not detected in any measurable amounts in any one of the three meal samples almost certainly precludes the possibility of a tannin being one of the factors responsible for the low feeding value of guar meal to poultry.

The presence of HCN in guar meal, although, according to Oke (1964), not in amounts high enough to create dangers of acute toxicity, was probably responsible in part for the increased requirement of dietary methionine (5.4) due to the involvement of cystine in the detoxification process of HCN. Neither the detection of low levels of trypsin inhibitor or haemagglutinating activities in guar meal is likely to affect the nutritive value of the meal to any great extent.

The gum contents of the three meal samples (Table 8.2) were estimated to be lower than those reported elsewhere (Nagpal et al., 1971). In view of the problems associated with the estimation of this fraction the values obtained may be underestimates.

The finding that guar meal possesses a saponin fraction (Table 8.3) is noteworthy and could be of great nutritional significance. Because there appears to be no other reports on the presence of a saponin fraction in guar no evidence other than the results of the biological trials during this investigation is available. The

comparative values for typical alfalfa meal saponins are included in Table 8.3. In view of the similarly adverse effects of feeding diets containing high levels of alfalfa meal and guar meal to poultry and their relative saponin contents there is a strong cause to believe that of a saponin more toxic than that in alfalfa is present in guar meal.

Furthermore, the lower "saponin" contents of ethanol-extracted GM-3 and NaOH-extracted GM-3, coupled with the improved performance of chicks fed diets containing these meals and the higher NPU values and ME values obtained for the two products (6.5 and 7.6), provide additional experimental evidence for the presence of a toxic saponin in guar meal.

The relatively lower saponin contents of GM-2 (Table 8.3) may also in part explain its better NPU value for chicks (6.7).

9. THE VALUE OF GUAR MEAL IN POULTRY DIETS

9. The value of guar meal in poultry diets

Guar meal is of interest as an ingredient in poultry diets for several reasons; it is available and is likely to continue to be available in commercial quantities; it has a fairly high protein content with an amino acid spectrum not dissimilar to that of soya bean meal; it is a by product and, as such, is not consumed by the human population. Guar meal presents no obvious storage, transhipment or mixing problems and does not require grinding before compounding into diets. The ash and fibre contents are reasonably low and would cause few if any problems to specifications if guar meal were introduced into poultry diets.

As is the case with most legumes the protein in guar meal contains relatively small amounts of methionine and cystine, particularly when the high requirements for these amino acids by poultry are considered. When guar meal-containing diets are fed to poultry the improvement in protein utilisation as a result of methionine supplementation has been amply demonstrated in the course of this study (5.4, 6.3, 6.4 and 6.5). The higher protein quality of guar meal sample 2 (GM-2) was attributed, at least in part, to the higher methionine content of its protein. This quite considerable variation in methionine levels between samples may mean that there is scope for plant geneticists to manipulate the amino acid make-up of the seed so that the methionine levels are better suited to poultry diet specifications. However, it is probably true to say that, even if "improved" strains of guar are developed, inclusion of guar meal in poultry diets at worthwhile inclusion rates will require the addition of methionine and thereby add to the cost of the diet. The demand for methionine may also be higher if the guar meal contains hydrocyanic acid due to the involvement of cystine in the

detoxification reactions. At the moment, synthetic methionine is readily available and invariably used in poultry diets so its requirement does not impose any constraint on the use of guar meal.

As a result of this study and research carried out elsewhere, it seems that the main constraint to the acceptance of guar meal as a component in poultry diets lies in the presence of certain antinutritional substances. These factors are undoubtedly responsible for the poor performance of both chicks (4.) and laying hens (5.) when appreciable amounts of guar meal are included in their diets. The identification and removal of these factors has occupied the major part of this study.

Although low levels of trypsin inhibitor activity were detected in vitro it is doubtful if its effect is of any nutritional significance. Small improvements in nutritive value were often detected after heat treatment (4.2 and 6.3) but there seems little justification for attributing those to the destruction of a heat labile proteolytic inhibitor. The content of tannins was so low that, despite the difficulty in their measurement in vitro, they were considered highly unlikely to be a factor affecting the nutritional quality of guar meal. The hydrocyanic acid and haemagglutinin contents of guar meal are likely only to be minor factors affecting the feeding value of the meal.

Undoubtedly a major factor influencing the value of guar meal for poultry is the residual gum. That guar gum at inclusion levels of as low as 10g/kg has a profoundly detrimental effect on bird performance as a result of reduced utilisation of the diet, is apparent (6.8 and 7.8). Most of the major components of the diet appear to be affected. This means that if 1kg guar meal contains 100g residual gum its inclusion at 100g/kg in poultry diets will be likely to reduce performance markedly. A concentration of

residual gum in the meal of this order seems reasonable and, judged by the proximate analysis of the seed components, may well be an underestimate (2.1 and 2.3).

The problems created by the residual gum, therefore, are a major constraint on the use of guar meal in poultry diets. It may be that the residual gum, which, it must be remembered, has valuable properties elsewhere, could be reduced to nutritionally tolerable proportions by the application of improved processing techniques during the milling of the seeds and the separation of the gum-containing endosperm from the other components. Whether the expense of increasing the efficiency of this operation can be justified in terms of the increased value of the meal and an increased yield of gum must rest with the judgement of someone else.

The adverse dietary effects of the gum on the utilisation of nutrients can, to a large extent, be overcome by the addition of certain enzyme preparations. Rhozyme HP-150, MKC hemicellulase and Betaganase M were all successful in improving the utilisation of guar meal (4.3, 4.4, 6.5, 6.6, 6.7, 7.5 and 7.7). These enzymes presumably act on the glycosidic bonds of the gum polysaccharides reducing them to oligo- and mono-saccharides which contribute to rather than reduce the value of the meal for poultry (7.8). In view of the availability commercially of these enzyme preparations and the tiny amounts required to hydrolyse the dietary gum the costs involved need not be a constraining factor.

Indirect and, hence, tentative evidence exists for the presence in guar meal of a toxic saponin, the removal or inactivation of which could represent a major advance in having the meal accepted as a high value ingredient for poultry diets. The bitter taste, the production of foam with water, the production of bloat in cattle and

the reduction of food intake by poultry are all characteristics of saponins and properties of guar meal. The most convincing proof of the existence of a saponin is contained in the experiments which studied the effect of dietary cholesterol on the utilisation of guar meal-containing diets (4.4, 5.3 and 6.7). Cholesterol is well known to overcome the adverse effects of dietary saponin inclusion although the mechanism is far from being understood. Attempts to quantify the saponin content of guar meal indicate that the levels may be quite high - about four times the amount in alfalfa (8.6). However, since the procedure was based on using soya bean saponin as the standard and the reaction is known to be dependent on the nature of the aglycone linkage to the carbohydrate moiety, the data is being treated with caution. Extraction of part of the saponin at least with ethanol and with sodium hydroxide obviously offers explanations for the improved performances of birds fed diets containing these extracted meals (6.5 and 7.6). It is possible that part of the improvement in nutritive value as a result of enzyme treatment of guar may have arisen as a result of cleavage of the sapogenin-carbohydrate bond of the saponin. The physiological effect of saponins is known to depend very much on this linkage as well as the nature of the aglycone.

Definitive proof awaits the isolation and characterisation of a physiologically active saponin. In the short term it is difficult to recommend a suitable course of action to the nutritionist. Dietary addition of cholesterol is not practical, least of all from an economic aspect. Extraction with ethanol has commercial possibilities although, unless its efficiency can be improved, seems likely only to reduce rather than remove the level of saponin. However, if some therapeutic or other industrial use could be found for the saponin, ethanol extraction could be the method of choice.

Perhaps a cheaper way of desaponising the meal could be its extraction with dilute alkali. An advantage of a process based on alkali would be the removal of any remaining gum. The disadvantages of alkali extraction appear to be a considerable loss of yield and the unlikely possibility of recovering the saponin. Perhaps the best long-term solution may be in the ability to breed low-saponin varieties of guar; the low level of saponin found in GM-2 would appear to indicate that this approach may be feasible.

The time for acceptance of guar meal as an ingredient suitable for inclusion at appreciable levels in poultry diets has not yet arrived. However, the identification and removal of the saponin and the removal of as much gum as possible would appear to leave a product of high nutritive value for poultry.

10. REFERENCES

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II. APPENDICES

APPENDIX A. METHODS OF ANALYSIS

A1. Chemical analysis

Conventional methods described by the Association of Official Agricultural Chemists (AOAC, 1965) have been used for estimation of the following constituents in samples of guar meal, feed ingredients and diets:

Ash (Total minerals)

Acid-insoluble ash

Crude fibre

Crude protein

Ether extract

Hydrocyanic acid

Moisture

Tannins

True protein

Amino acids

Samples were hydrolysed by refluxing in 6N hydrochloric acid for 20 hours in an atmosphere of nitrogen and the hydrolysate applied to a Technicon type NC-6 amino acid analyser. The resin support was Technicon chromobeads type A.

Available carbohydrates

The available carbohydrates contents of guar meal samples and diets were determined after enzymic hydrolysis with takadiastase and hydrolysis of the soluble fraction with 70% H_2SO4. The resultant sugars were measured, after reaction with orcinol, using an autoanalyser with glucose as standard (Shannon, 1972).

Trace minerals

The mineral analyses of guar meal samples were carried out using a Varian Techtron atomic absorption spectrophotometer.

Cystine and methionine

Cystine and methionine were determined in hydrolysates as cysteic acid and methionine sulphone respectively after oxidation of the protein with performic acid (Moore, 1963).

Plasma cholesterol

The plasma cholesterol contents were estimated by an automated version of the Boehringer enzymic method described by Röeschlau et al. (1974).

A2. Statistical analysis of data

Experiments described in 4.1, 4.2, 4.3 and 5.4 were analysed using 'GENSTAT' statistical programme developed by the Agricultural Research Council's Rothamsted Experimental Station. Other analyses were carried out manually on a Casio AL 2000 electronic calculator using analysis of variance methods (Steel and Torrie, 1960).

APPENDIX B. TABLES OF COMPOSITIONS

B1 Compositions of vitamin and mineral premixes for use in broiler chicks and laying hens rations

Supplied per kg diet	Broiler chicks	Laying hens
Vitamin mix		
Vitamin A, IU	2000	6000
Vitamin D_3, IU	600	800
Vitamin E, IU	25	25
Menaphthone, mg	1.3	1.3
Riboflavin, mg	4	4
Nicotinic acid, mg	28	28
Pantothenic acid, mg	10	10
Biotin, µg	50	-
Maize meal to make	2.5g	2.5g
Mineral mix		
Copper (as cupric sulphate), mg	3.5	3.5
Iodine (as potassium iodate), mg	0.4	0.4
Iron (as ferrous sulphate), mg	80	80
Magnesium (as magnesium carbonate), mg	300	300
Manganese (as manganous carbonate), mg	100	100
Zinc (as zinc oxide), mg	50	50
Maize meal to make	2.5g	2.5g

Composition of mineral premix used in the NPU experiments

Mineral	g/kg of diet
$CaCO_3$	22.715
$K\ H_2PO_4$	12.567
$CaHPO_4.2H_2O$	12.508
NaCl	7.144
$Mg\ SO_4$	2.985
$Fe\ SO_4.7H_2O$	0.495
$Zn\ SO_4$	0.300
$Mn\ SO_4.\ 5H_2O$	0.023
$Na_2MoO_4.2H_2O$	0.011
KI	0.011

B3 Composition of vitamin premix used in the NPU experiments

Vitamin	Amount per kg of diet
Vitamin A, I.U.	6000
Vitamin D_3, I.U.	600
Vitamin E, I.U.	25
Ascorbic acid, mg	150
Nicotinic acid, mg	100
Thiamine HCl, mg	25
Calcium pantothenate, mg	20
Riboflavin, mg	15
Pyridoxine HCl, mg	5
Menaphthone, mg	4
Folic acid, mg	4
Biotin, mg	0.5
Vitamin B_{12}, µg	20
Glucose to make	2.5 g

B4 Per cent composition of broiler mash

Maize meal	30.00
Wheat meal	24.65
Soya bean meal	26.00
Meat-and-bone meal	10.00
Maize germ meal	5.00
Maize oil	2.50
$CaHPO_4$	0.50
$CaCo_3$	0.40
Vitamin premix *	0.25
Mineral premix *	0.25
NaCl	0.20
DL-Methionine	0.20
Choline chloride	0.05

Calculated analysis

Crude protein %	22.0
Calcium %	1.1
Phosphorus %	0.7
ME (kcal/g)	2.9

* Compositions of vitamin and mineral premixes are set out in Appendix B1.

B5

Analyses of diets used in broiler experiment 1

(per cent, air-dry basis)

	Starter diet							
	1A	1B	1C	1D	1E	1F	1G	1H
Dry matter	87.8	86.9	87.1	87.9	87.5	87.6	87.3	87.8
Crude protein (Nx6.25)	22.1	22.1	21.9	22.3	22.1	22.5	22.4	22.4
Ether extract	6.8	8.0	8.5	7.7	8.0	8.4	8.1	7.9
Total ash	8.8	6.7	6.1	6.3	6.6	6.4	6.4	6.6
Calcium	1.2	1.2	1.0	1.2	1.2	1.1	1.2	1.0
Phosphorus	0.72	0.80	o.75	o.80	0.78	0.79	0.81	0.76
Methionine (calculated)	0.5	0.5	0.5	0.5	0.5	1.0	1.0	1.0
ME* (kcal/g, calculated)	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
	Finisher diet							
Dry matter	87.2	87.2	87.5	87.8	87.8	88.0	88.0	88.1
Crude protein	18.9	18.5	19.6	19.1	19.0	19.7	19.2	19.7
Ether extract	6.2	6.5	7.7	8.2	8.0	8.6	8.7	8.4
Total ash	6.6	7.0	6.6	6.3	6.3	6.7	6.5	6.8
Calcium	1.0	1.1	1.1	1.1	0.9	1.0	1.0	1.0
Phosphorus	0.8	0.72	0.71	0.74	0.7	0.75	0.76	0.77
Methionine (calculated)	0.4	0.4	0.4	0.4	0.4	0.9	0.9	0.9
ME* (kcal/g, calculated)	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0

* Assuming a ME value of 2000kcal/g of guar meal.

B6 Chemical composition of water-extracted, ethanol-extracted and sodium hydroxide-extracted guar meal samples

Constituents	GM-1 water-extracted	GM-3 ethanol-extracted	GM-3 sodium hydroxide-extracted
Dry matter %	92.5	91.7	92.6
Crude protein %	46.3	44.3	36.7
Oil %	5.9	2.9	5.9
Available carbohydrate %	6.5	13.1	16.1
Ash %	4.2	5.0	5.4
Calcium %	0.16	0.25	0.27
Phosphorus %	0.32	0.49	0.50
Amino acids (g/16gN)			
Alanine	3.31	3.65	3.86
Arginine	12.81	12.25	11.92
Aspartic acid	9.04	10.11	9.62
Glutamic acid	15.33	19.50	18.86
Glycine	4.83	5.08	5.20
Histidine	2.60	2.39	2.77
Isoleucine	3.17	2.98	3.73
Leucine	6.21	5.75	6.64
Lysine *	3.98	4.13	4.38
Methionine *	1.36	1.12	1.55
½ Cystine * [1]	1.39	1.19	1.02
Phenylalanine	3.88	3.88	3.96
Proline	2.60	3.70	3.09
Serine	4.06	4.53	4.76
Threonine **	3.06	3.20	4.31
Tryptophan **	1.70	1.80	1.19
Tyrosine	3.73	3.41	3.03
Valine	3.58	3.68	4.43

* Determined after oxidation according to Moore (1963)

** Determined by pronase method (Holz, 1972).

[1] Cystine + cysteine expressed as ½ cystine

B7 Composition and analyses of diets fed to birds in the rearing stage (g/kg)

	Rearing diets	
	Starter (0 to 6 weeks)	Grower (7 to 19 weeks)
Ingredients		
Ground wheat	654.0	754.0
Soya bean meal	280.0	180.0
Maize oil	24.5	25.0
$CaHPO_4$	20.0	20.0
$CaCO_3$	7.5	7.5
NaCl	5.0	5.0
Vitamin premix*	2.5	2.5
Mineral premix*	2.5	2.5
L-Lysine	2.0	2.5
DL-Methionine	2.0	1.0
Analyses		
Dry matter	869	875
Crude protein	198	158
Ether extract	28	26
Ash	59	58
Calcium	11	10
Phosphorus	61	55

* The compositions of vitamin and mineral premixes are set our in Appendix B1.

Apparent metabolisability* of amino acids after admixture of guar gum or a mixture of galactose and mannose to a basal diet

	Amino acid in basal diet, 20A** (%)	Apparent metabolisability of amino acids						
		20A** (%)	20B*** (%)	R†	20C*** (%)	R†	20D**** (%)	R†
Alanine	0.98	77.7±2.6	63.0±3.9	81.0	56.9±8.9	73.2	76.4±1.1	98.3
Aspartic acid	1.50	75.5±2.6	63.0±3.8	83.4	59.2±7.2	78.4	72.9±1.0	96.6
Arginine	1.16	85.7±1.6	78.5±2.4	91.6	77.2±3.7	90.1	83.9±0.2	97.4
Glutamic acid	2.93	84.1±2.1	76.4±2.8	90.8	69.0±5.5	82.0	81.1±1.6	96.4
Histidine	0.42	79.9±2.2	70.1±3.2	87.7	65.4±6.4	81.9	79.3±0.9	99.2
Isoleucine	0.67	79.5±2.1	66.9±3.1	84.2	62.3±6.9	78.4	76.7±0.8	96.5
Leucine	1.44	83.0±2.0	71.8±3.0	86.5	67.6±5.9	81.4	81.1±0.4	97.7
Lysine	0.97	79.6±2.1	69.6±3.0	87.4	66.3±5.7	83.3	77.6±0.8	97.5
Methionine	0.34	86.2±1.7	74.3±2.7	86.2	71.5±5.5	82.9	82.2±0.8	95.4
Cystine	0.22	82.0±2.3	62.6±4.9	76.3	58.7±9.5	71.6	79.3±1.2	96.2
Phenylalanine	0.77	83.1±1.8	72.7±3.4	87.5	69.1±5.9	83.2	81.4±0.8	97.9
Proline	0.84	77.0±3.2	64.2±4.9	83.4	57.8±9.2	75.1	74.6±1.6	96.9
Serine	0.82	78.5±2.5	65.8±3.9	83.8	60.1±7.0	76.6	75.7±1.3	96.4
Threonine	0.56	71.3±3.1	53.8±4.4	75.5	48.7±9.2	68.3	67.3±1.9	94.4
Tyrosine	0.56	79.5±2.4	65.0±4.2	81.8	61.0±7.6	76.7	75.7±2.2	95.2
Valine	0.83	77.0±2.5	64.0±3.5	83.1	59.0±7.0	76.6	73.1±1.8	94.9
Mean ± SE		80.0±1.0	67.6±1.56	84.4 ±1.11	63.1±1.73	78.7 ±1.34	77.4±1.06	96.7 ±0.32

* Amino acid intake (g/g food) - amino acid in droppings (g/g food)÷ amino acid intake (g/g food)

** Identical to diet 3A (Table 4.10)

*** Diets 20B and 20C contained 2.5 and 5.0% guar gum (Sigma Chem.) respectively

**** Contained 5% of a mixture of D-Galactose and D-Mannose, 35:65 parts respectively

† Per cent of basal

APPENDIX C. TABLES OF ANALYSIS OF VARIANCE

In the following analysis of variance (ANOVA) tables

df	=	degrees of freedom
F	=	variance ratio
MS	=	mean square
ns	=	not significant
*	=	significant at P 0.05
**	=	significant at P 0.01
***	=	significant at P 0.001

C1

ANOVA Table - Broiler experiment 2

		Initial weight		Weight gain		Food intake		FCE	
Source of variation	df	MS	F	MS	F	MS	F	MS	F
Guar meal	3	1.162	< 1 ns	16844.5	64.848***	7149.9	8.092**	2.284	20.245***
Vitamins/and methionine	3	1.127	< 1 ns	226.0	< 1 ns	2035.6	2.304 ns	0.466	4.133*
Guar meal x vitamins	9	1.855	1.345 ns	180.0	< 1 ns	1120.7	1.268 ns	0.095	< 1 ns
Residual	32	1.379		259.8		883.5		0.112	
Total	47	1.440		1300.9		1402.5		0.270	

C2

ANOVA Table - Broiler experiment 3

		Weight-gain (29-56d)		Food intake (29-56d)		FCE	
Source of variance	df	MS	F	MS	F	MS	F
Block (B)	1	207.679	< 1 ns	5619.97	< 1 ns	0.166	< 1 ns
Guar meal level (GM)	3	94199.523	48.11 ***	54924.10	6.37 **	42.912	56.24 ***
Enzyme (E)	2	44113.537	22.53 ***	69209.15	8.03 **	11.894	15.58 ***
Diet form (F)	1	65913.788	33.66 ***	342584.00	39.74 ***	1.343	1.76 ns
GM x E	6	4167.936	2.13 ns	1340.14	< 1 ns	2.564	3.36 *
GM x F	3	2042.413	1.04 ns	2739.36	< 1 ns	0.885	1.16 ns
E x F	2	138.998	< 1 ns	6679.22	< 1 ns	0.274	< 1 ns
GM x E x F	6	2379.833	1.21 ns	10530.51	1.22 ns	0.477	< 1 ns
Covariance	1	480.223		2683.87		0.217	
Residual	22	1957.935		8619.19		0.763	

C3

ANOVA Table - Broiler experiment 3

(Carcass composition)

		Moisture		Protein		Fat		Minerals	
Source of variance	df	MS	F	MS	F	MS	F	MS	F
Enzyme (E)	2	3.258	2.20 ns	0.165	< 1 ns	3.818	1.95 ns	0.045	1.94 ns
Diet form (F)	1	7.053	4.78 *	0.025	< 1 ns	7.963	4.08 ns	0.003	< 1 ns
Guar meal level (GM)	3	1.765	1.19 ns	0.148	< 1 ns	0.517	< 1 ns	0.033	1.40 ns
E x F	2	0.169	< 1 ns	0.395	1.35 ns	0.524	< 1 ns	0.021	< 1 ns
E x GM	6	0.889	< 1 ns	0.290	< 1 ns	1.640	< 1 ns	0.030	1.28 ns
F x GM	3	0.680	< 1 ns	0.284	< 1 ns	0.721	< 1 ns	0.008	< 1 ns
E x F x GM	6	0.614	< 1 ns	0.388	1.33 ns	0.868	< 1 ns	0.067	2.87 ns
Blocks	1	0.017	< 1 ns	0.035	< 1 ns	0.038	< 1 ns	0.041	1.75 ns
Residual	23	1.476		0.292		1.952		0.023	
Total	47	1.366		0.282		1.709		0.030	

C4

ANOVA Table - ME Experiment 5

Balance period of 3 days

		Food intake		Apparent DM metabolisability		Nitrogen retention		ME_n of diet	
Source of variation	df	MS	F	MS	F	MS	F	MS	F
Diets	5	537	< 1	250.55	15.2***	233.25	11.3***	456925	20.3***
Error	24	2063		16.44		20.58		22506	
Total	29	1800		56.80		57.25		97405	

Feeding period of 7 days

		Weight gain		Food intake		FCE		W/F Ratio	
Source of variation	df	MS	F	MS	F	MS	F	MS	F
Diets	5	7920	5.58***	4889	< 1	333.14	16.28***	65.24	9.14***
Error	24	1419		6960		20.46		7.14	
Total	29	2540		6603		74.37		17.15	